AMNIOTIC MEMBRANE TRANSPLANTATION FOR OCULAR SURFACE DISORDERS

THESIS

FOR

MASTER OF SURGERY

[OPHTHALMOLOGY]

M.L.B. Medical College, Jhansi

BUNDELKHAND UNIVERSITY

JHANSI (U.P.)

2004

MONIKA GUPTA

DEDICATED

TO

MY PARENTS

DEPARTMENT OF OPHTHALMOLOGY
M. L. B. MEDICAL COLLEGE
JHANSI

CERTIFICATE

Certified that the research work entitled ***"Amniotic Membrane Transplantation For Ocular Surface Disorders"*** which is being submitted as thesis for M.S. (ophthalmology) examination of Bundelkhand University, 2004 by ***Dr. Monika Gupta*** has been carried out in the department of ophthalmology, M.L.B. Medical college Jhansi.

She has put in the necessary stay in this department as per university regulations.

[Dr. B.S. JAIN]
M.S.
Head,
Department of Ophthalmology,
M.L.B. Medical College,
Jhansi.

DEPARTMENT OF OPHTHALMOLOGY
M. L. B. MEDICAL COLLEGE
JHANSI

CERTIFICATE

Certified that the research work entitled *"**Amniotic Membrane Transplantation For Ocular Surface Disorders**"* was conducted by ***Dr. Monika Gupta*** herself, under my direct supervision and guidance. The investigations, techniques and statistics mentioned in the thesis were actually undertaken by the candidate herself and observations have been checked by me regularly.

[Dr. B.S. JAIN]
M.S.
Head,
Department of Ophthalmology,
M.L.B. Medical College,
Jhansi.
(GUIDE)

DEPARTMENT OF OPHTHALMOLOGY
M. L. B. MEDICAL COLLEGE
JHANSI

CERTIFICATE

Certified that the research work entitled *"**Amniotic Membrane Transplantation For Ocular Surface Disorders**"* was conducted by ***Dr. Monika Gupta*** herself, under my direct supervision and guidance. The investigations, techniques and statistics mentioned in the thesis were actually undertaken by the candidate herself and observations have been checked by me regularly.

[Dr. V.K. Misuriya]
M.S.
Assistant Professor,
Department of Ophthalmology,
M.L.B. Medical College,
Jhansi.
(CO-GUIDE)

DEPARTMENT OF OPHTHALMOLOGY
M. L. B. MEDICAL COLLEGE
JHANSI

CERTIFICATE

Certified that the research work entitled *"**Amniotic Membrane Transplantation For Ocular Surface Disorders**"* was conducted by **Dr. Monika Gupta** herself, under my direct supervision and guidance. The investigations, techniques and statistics mentioned in the thesis were actually undertaken by the candidate herself and observations have been checked by me regularly.

[Dr. **R.K. AGARWAL**]
M.D.
Head of the Department,
Department of Microbiology,
M.L.B. Medical College,
Jhansi.
(CO-GUIDE)

Acknowledgement

It is a pleasure to avail this golden opportunity to express my sincere regards and thanks to my esteemed guide and revered teacher Dr. B.S. Jain MS, Head, Department of Ophthalmology, MLB Medical College, Jhansi. Although no words can express the depth of my sense of gratitude, his firm support, constant encouragement & unrestrained accessibility provided the morale needed for undertaking such a project. I will always try to follow his punctuality, meticulous attention to details, methodical approach and his unflagging zeal for his work. He is always receptive to new ideas and it was on his valuable initiation and advice that I commenced this study. I will always remain indebted to him for his ingenious guidance and gracious direction.

I am also indebted to Dr. V.K. Misurya, Associate Professor, Department Of Ophthalmology, for his precious guidance, expert advice and kind supervision which encouraged me in the completion of this work.

I am also very grateful to Dr. R.K. Agarwal MD, Head, Department of Microbiology, for his invaluable guidance and stimulating

exchange of ideas, especially regarding the preparation and preservation of amniotic membrane.

I am immensely thankful to my respected parents, my brother Vipul, sister-in-law Abha, my fiancée Dr. Ashutosh and my in-laws for their numerous sacrifices, boundless co-operation, constant support and inspiration, which have kept me from faltering throughout this endeavor.

I also extend my sincere thanks to my seniors, colleagues, friends and juniors especially, Dr. Anant, Dr. Charu, Dr. Deepali, Dr. Mamta, Dr. Ibemhal and Dr. Niti for their invaluable help without which this work would have been a lot more difficult to complete. Last but not the least, I thank the subjects of this study without whose co-operation and patience this study could not have been accomplished.

For designing of this manuscript, I am thankful to Mr. Vinod Raikwar (V.K. Graphics).

Monika Gupta

Dated : 23/01/2004

Dr. Monika Gupta

CONTENTS

S.NO.	DESCRIPTION		PAGE NO.
1.	INTRODUCTION	...	1 - 4
2.	REVIEW OF LITERATURE	...	5 - 32
3.	AIM & OBJECTIVES	...	33
4.	MATERIAL AND METHODS	...	34 - 41
5.	OBSERVATIONS	...	42 - 52
6.	DISCUSSION	...	53 - 61
7.	SUMMARY AND CONCLUSION	...	62 - 64
8.	BIBLIOGRAPHY	...	65 - 78

INTRODUCTION

Introduction

The cornea, the conjunctiva consisting of bulbar, fornical and palpebral conjunctiva and the intervening transition area, known as limbus, comprise the tissues at the ocular surface. Functionally, all three regions of the epithelium support the tear film and protect against fluid loss and pathogen entrance. The limbus, a narrow 1.5-2mm band of tissue straddling the cornea and the conjunctiva, is the site of the corneal epithelial stem cells. Stem cells are responsible for the ultimate cellular replacement and tissue regeneration in all self-renewing tissue.

Damage to these cells from certain systemic inflammatory diseases or primary ocular diseases or trauma may lead to various Ocular Surface Disorders. Loss of stem cells may result from chemical / thermal injuries Stevens-Johnson syndrome, multiple surgeries or cryotherapies to the limbal region, contact lens-induced keratopathy or toxic effects from lens-cleaning solutions, neurotrophic keratopathy (neuronal or ischemic), peripheral corneal ulcerative keratitis,pterygium and pseudopterygium. The result is breakdown of the ocular surface and corneal epithelial defects that may become chronic if the normal epithelialization process fails. Conjunctival epithelium may replace corneal epithelium resulting in loss of corneal transparency. Chronic inflammation may then occur characterized by neovascularization, corneal scarring and opacification,

corneal thinning, and possible corneal perforation, all of which may lead to loss of visual acuity.

Ocular surface disorders caused by various conditions of the cornea and conjunctiva are still difficult to treat. Persistent corneal epithelial defect, or irregular surface of the cornea from bullous or band keratopathy can cause not only irritation and pain to the patient but also reduce the protective mechanism and lead to the infection of the cornea. Current medical treatments include topical artificial tears, lubricants, and experimental trials of fibronectin, insulin-like growth factor type I and substance P, or nerve growth factor. **When these medical therapies fail, patching, scleral contact lens, cyanoacrylate glue, conjunctival flap, and tarsorrhaphy are considered**. Punctal occlusion may be beneficial. Therapeutic contact lenses have been shown in some cases to be effective therapy for persistent epithelial defect but carry the increased risk of infection. Conjunctival flap will change the clear cornea to vascularization. More invasive surgical therapies include temporary or permanent tarsorrhaphy but its efficacy is limited by the ability of the corneal wound to heal, and affects the appearance of the patient. Lysis adhesion from the scar and symblepharon or wide excision of the conjunctival mass still needs a good graft in terms of ease of application, good cosmetic results, and prevention of tissue adhesion.

Recently, amniotic membrane transplantation (AMT) has been successfully used to treat persistent corneal epithelial defects and ulcers from different causes, and for corneal and conjunctival surface reconstruction for a variety of ocular surface disorders. Use of human amniotic membrane for transplantation may be an alternative or adjunctive therapy. In comparison with other biologic tissues used as reconstructive grafts, the amniotic membrane presents some undoubted advantages; It is thinner and better tolerated by the patient and it never becomes necrotic; It is not a substitute of the conjunctiva, but rather a substrate where conjunctival cells migrate and regenerate, forming new and healthy tissue.

Over the last several years there has been a tremendous and growing demand for amniotic tissue to treat conditions such as Stevens-Johnson disease, ocular surface disorders, chemical and thermal burns, deep corneal ulcers, pterygium, bullous keratopathy and other ophthalmic indications. Recently, amniotic membrane transplantation (AMT) has been used in many different types of reconstructive surgery. AMT became important because of its ability to diminish the occurrence of adhesions and scarring, its ability to enhance wound healing and epithelialisation, and its antimicrobial potential

The transplantation of human amniotic membrane has been added to the therapeutic armamentarium. Certain characteristics make the

amniotic membrane ideally suited to its application in ocular surface reconstruction. It can be easily obtained and its availability is nearly unlimited.

In this study, the effectiveness of amniotic membrane transplantation for various ocular surface disorders has been studied prospectively.

REVIEW OF LITERATURE

Review of Literature

An ideal graft for use in ocular surface reconstruction would not only, to a maximum, promote healing while minimizing scarring, but would also yield cosmetically acceptable results and be relatively easy to perform. Human amniotic membrane clearly has shown promise along these lines. Amniotic membrane transplantation has been successfully used in patients with persistent epithelial defects unresponsive to medical treatment, and as an alternative to conjunctival flaps, botulinum toxin injection, or tarsorrhaphy. The amniotic membrane, by virtue of its transparency, allows the patient navigational vision and is particularly useful if the affected eye is the better seeing eye. The use of more than one layer may be effective in covering ulcers with substantial stromal depth. Amniotic membrane has been used as an alternative to conjunctival autograft during the removal of pterygia. The recurrence rate of pterygium after AMT (10.9% for primary pterygia) was lower than the bare sclera technique (45%), but higher than autologous conjunctival graft (2.6%). Multiple surgical approaches have been used to treat pterygium. Although conjunctival autograft is considered to be the most efficient, AMT appears to be a reasonable option in cases with diffuse conjunctival

involvement and patients in whom the bulbar conjunctiva must be preserved for a prospective glaucoma filtering procedure.

AMT has been successfully used in the treatment of recurrent pterygium associated with severe symblepharon and diplopia. AMT has been used successfully in the reconstruction of conjunctival defects created during surgical removal of large conjunctival lesions.

Corneal stem cell deficiency is associated with conjunctivalisation of the cornea and can be complicated with persistent epithelial defects, vascularisation, scarring, calcification, ulceration, melting, and perforation of the cornea. Patients with these abnormalities are poor candidates for conventional corneal transplantation. Lamellar or penetrating keratoplasty provides only a temporary replacement of the host's corneal epithelium and does not permanently restore limbal function. In cases with diffuse corneal stem cell deficiency, limbal transplantation (allo or auto) is now considered essential for corneal surface reconstruction. AMT combined with limbal transplantation has been successfully used in patients with diffuse limbal stem cell deficiency and severe ocular surface disease, including Stevens-Johnson syndrome, advanced ocular cicatricial pemphigoid, chemical and thermal burns. Alternatively, autologous limbal-corneal epithelium can be cultured on amniotic membrane and used for corneal surface reconstruction

AMT has been successfully used in patients to treat myopic regression with corneal opacity after photorefractive keratectomy (PRK) in high myopia. Excessive corneal haze and myopic regression are associated with excessive healing response, which might be inhibited by amniotic membrane. In rabbits the corneal haze was reduced by AMT in excimer laser photoablation.

In 1910 Davis was the first to report the use of fetal membranes as surgical material in skin transplantation. Since then the use of amniotic membrane in surgery has been expanded..Both fresh and preserved human amniotic membrane have been widely used effectively as a biological dressing for acute burns (Bose 1979; Sharma et al. 1985; Talmi et al. 1990; Ramakrishnan et al. 1997) , skin ulcers (Somerville 1982; Shun et al. 1983) and abdominal wounds (Silverton et al. 1979) to relieve pain, promote epithelial healing and decrease infection rate of the wound. The amniotic membrane has also been used as a graft for burned skin (Waikakul et al. 1990; Subrahmanyam 1995), reconstruction in otolarynx (Zohar et al. 1987), and repair omphalocele (Yokomari et al. 1992). It has also been reported to prevent tissue adhesion in surgeries of head, abdominal and pelvic cavity (Trelford-Sauder et al. 1978; Young et al 1991; Rennekampff et al. 1994).

Human amniotic membrane was first used as a graft for treating conjunctival defects (DeRoth 1940) and pterygium (Panzardi 1947) since 1940. In 1993, preserved human amniotic membrane was successfully used in corneal surface reconstruction in rabbit eyes by Kim and Tseng (1995). Since then, this technique has been applied as an adjunct in the treatment of many ocular surface disorders. In corneal surface reconstruction, the amniotic membrane is reported to have been used in persistent epithelial defects (Lee et al. 1997; Kruse et al. 1999; Azuara-Blanco et al 1999), combined with limbal transplantation in Steven-Johnson syndrome, and chemical burns (Tsubota et al 1996; Shimazaki et al. 1997; Tseng et al. 1998; Tsubota et al 1999), bullous keratopathy (Pires et al. 1999) and for reducing haze in following phototherapeutic keratectomy (PRK) (Wang et al. 1997; Choi et al. 1998).

Lee SH, Tseng SC. (1997) conducted a study to determine whether preserved human amniotic membrane can be used as an alternative substrate for treating persistent corneal epithelial defects with sterile ulceration. Amniotic membrane transplantation was performed in 11 eyes of 11 consecutive patients with corneal ulcers of different causes. Ten patients healed in 3.9 +/- 2.3 weeks ($P < .01$) without recurrence for 9.0 +/- 5.9 months. One patient failed to heal because of preexisting corneal perforation pursuant to severe rheumatoid arthritis. They

concluded that Amniotic membrane transplantation may be considered an alternative method for treating persistent epithelial defects and sterile ulceration that are refractory to conventional treatment and before considering treatment by conjunctival flaps or tarsorrhaphy.

Tseng SC, Prabhasawat P, Lee SH. (1997) conducted a study to determine whether preserved human amniotic membrane can be used to reconstruct the conjunctival defect created during surgical removal of a large lesion or during symblepharon lysis. They concluded that Amniotic membrane transplantation can be considered an alternative substrate for conjunctival surface reconstruction during removal for large tumors, disfiguring scars, or symblepharon, especially for those whose surrounding conjunctival tissue remains relatively normal. .the following table summarizes the study.

INDICATION	NO. OF CASES	SUCCESS	PARTIAL SUCCESS	FAILURE
To reconstruct the conjunctival defect created during surgical removal of a large lesion lysis	7	11(68.7%)	2 (12.5%)	3 (18.7%)
To reconstruct the conjunctival defect created during symblepharon lysis	9			

Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D. (1998)

Did a study to examine whether amniotic membrane transplantation (AMT), in preparing the perilimbal stroma, enhances the success of allograft limbal transplantation (ALT). Thirty-one eyes of 26 consecutive patients had cytologically proven limbal deficiency resulting from chemical burns (14 eyes); Stevens-Johnson syndrome, toxic epidermal necrolysis, or pseudopemphigoid (5 eyes); contact lens-induced

keratopathy (3 eyes); aniridia (3 eyes); multiple surgical procedures (2 eyes); atopy (2 eyes); or an unknown cause (2 eyesThey conducted that for partial limbal deficiency with superficial involvement, AMT alone is sufficient and hence superior to ALT because there is no need to administer cyclosporine. For total limbal deficiency, additional ALT is needed, and AMT helps reconstruct the perilimbal stroma, with reduced inflammation and vascularization, which collectively may enhance the success of ALT. The following table summarizes the study.

INDICATIONS	NO. OF CASES	SURGICAL PROCEDURE	SUCCESS RATE
Mild limbal stem cell deficiency	10	AMT	93.5%
Moderate limbal stem cell deficiency	7	AMT + ALT	
Severe limbal stem cell deficiency	14	AMT + ALT + Penetrating keratoplasty	

Shimazaki J, Shinozaki N, Tsubota K. (1998) studied whether the treatment of recurrent pterygium associated with symblepharon requires both suppression of fibrosis and reconstruction of limbal barrier. To achieve this, they conducted the study in which human amniotic

membrane was transplanted and limbal autografts performed. Four patients with severe symblepharon resulting from multiple surgeries for pterygium were treated.the amniotic membrane was placed on the sclera, and a limbal autograft transplantation was performed using limbal tissues taken from the affected eye. Recurrence of symblepharon was not observed in any of the patients and significant suppression of the subconjunctival fibrosis was achieved. Ocular movement improved in all cases. Complete remission of pterygium regrowth occurred in three cases, and a slight (about 1 mm) recurrence occurred in one case. The limbal donor site showed the presence of mild depressions without the formation of pseudopterygium. They conducted that Transplantation of human amniotic membrane with a limbal autograft appears to be a promising surgical treatment for reconstructing the ocular surface in patients with recurrent pterygium associated with symblepharon.

Gris O, Guell JL, Lopez-Navidad A, Caballero F, Del Campo Z. (1999) Carried out the amniotic membrane implantation on 11 patients with different pathologies: three cases of limbal stem cell deficiency (caustication with failure of prior keratoplasty, congenital aniridia and post-radiotherapy keratopathy), one case with persistent neurotrophic corneal ulcer after prior keratoplasty, four cases with epithelial defect of long evolution, one case of extensive Salzmann's degeneration of the

cornea, and two cases after the resection of conjunctival tumour. In all transplanted patients the reabsorption of the amniotic membrane took place between the third and the fifth week. They conducted that the implantation of preserved human amniotic membrane can favour the recovery of a normal ocular surface in different pathologies, both in corneal and conjunctival lesions.

Gabric N, Mravicic I, Dekaris I, Karaman Z, Mitrovic S (1999) Sought to determine the efficacy of amniotic membrane transplantation (AMT) in the reconstruction of ocular surface. AMT was performed on 40 eyes with following indications: I, persistent corneal ulceration (n = 12); II, impending perforation (n = 6); III, persistent epithelial defect on the corneal graft (n = 6); IV, recurrent pterygia (n = 10), and V, risk of conjunctival scarring (n = 6). they concluded that AMT have shown to be effective in enhancing healing of the corneal defects, in prevention of symblepharon formation and recurrent pterygium ingrowth. In case of impending perforation, AMT alone was not a method of treatment but is useful as a first step procedure in preparing the eye for the corneal transplantation.the following table summarizes the study.

Group	Indication	No. of cases	Success rate
I	Persistent corneal ulceration	12	67%
II	Impending perforation	6	100%
III	Persistent epithelial defect on corneal graft	6	33%
IV	Recurrent pterygium	10	70%
V	Risk of conjunctival scarring	6	84%

Rakowska E, Zagorski Z, Kardaszewska A, Durakiewicz D (1999) Presented their experience with the amniotic membrane transplantation in severe corneal diseases. amniotic membrane transplantation was applied in 18 eyes (17 patients) with severe and persistent corneal diseases..They observed that amniotic membrane dissolved more quickly in a vascularized bed.They concluded that Amniotic membrane transplantation may be considered a good alternative method for treating severe corneal disorders that are refractory to conventional treatment. The following table summarizes the study.

Indication	No. of cases	Success rate
Perforated corneal ulcer	9	77.7%
Non perforated corneal ulcer	4	100%
Recurrent transplant disease	2	50%
Keratolysis	1	0%

Kruse FE, Rohrschneider K, Volcker HE (1999) conducted a study evaluate the efficacy of multilayer amniotic membrane transplantation for reconstruction of corneal epithelium and stroma in the context of deep corneal ulcers. Eleven consecutive patients with deep corneal ulcers refractory to conventional treatment; six patients had herpetic keratitis and five had other forms of neurotrophic keratitis. After multilayer amniotic membrane transplantation with kryopreserved human amniotic membrane, Integrity of corneal epithelium and stroma, opacification, and appearance of grafted membrane was observed during 12 months follow-up. Amniotic membrane transplantation markedly reduced ocular inflammation in all patients. They concluded that amniotic membrane transplantation allows corneal surface reconstruction in

patients with persistent epithelial defects. The multilayer technique is useful for treating deep corneal ulcers and even descemetoceles. Because the procedure results in stability of the ocular surface over a period of more than 12 months in most patients, it may be considered an alternative to conventional surgical techniques for ocular surface reconstruction.

Zhou S, Chen J, Xu L, Lin J, Huang T (1999 Sep) conducted a study to determine whether fresh human amniotic membrane can be used to reconstruct the conjunctival detect created during symblepharon lysis. Forty-two eyes of 39 consecutive patients with eye burns and Stevens-Johnson syndrome were randomized to accept fresh or preserved human amniotic membrane transplantation (AMT) during the period of severe scarring. During a mean follow-up of 11 months (range, 6 to 18 months), thirty-five patients (37 eyes) showed successful ocular surface reconstruction and resolution of motility They concluded that both fresh and preserved human amniotic membrane can be considered an ideal alternative substrate for conjunctival surface reconstruction during removal of severe symblepharon.

Meller D, Tseng SC (2000) Conducted a study to know whether amniotic membrane transplantation (AMT) is useful in preparing the perilimbal stroma to enhance the success of allograft limbal transplantation (ALT). They concluded that For partial LD with

superficial involvement, AMT alone is sufficient and hence superior to ALT because of no need for using cyclosporin A. For total LD, additional ALT is needed and AMT helps reconstruct the perilimbal stroma with reduced inflammation and vascularization, which collectively may enhance ALT success. The following table summarizes the study.

INDICATIONS	NO. OF CASES	SURGICAL PROCEDURE	SUCCESS RATE
Mild limbal deficiency	18	AMT	95.7%
Moderate limbal deficiency	13	AMT + ALT+ cyclosporin A	
Severe limbal deficiency	16	AMT + ALT + Penetrating keratopathy + cyclosporin A	

Pires RT, Chokshi A, Tseng SC (2000) conducted a study to determine if human amniotic membrane transplantation or limbal stem cell transplantation is effective to restore the corneal surface with partial or total limbal stem cell deficiency, respectively, caused by 5-fluorouracil (5-FU) toxicity after glaucoma surgeries.Amniotic membrane transplantation or conjunctival limbal autograft was performed for corneal surface reconstruction, respectively. For a period of 15 months of

follow-up, the visual acuity improved, and their corneal surfaces remained avascular, smooth, and without recurrence of limbal stem cell deficiency. They concluded that partial limbal stem cell deficiency can be treated with amniotic membrane transplantation alone, whereas limbal transplantation must be considered as an alternative for total limbal stem cell deficiency to restore the corneal surface integrity and vision.

Meller D, Pires RT, Mack RJ, Figueiredo F, Heiligenhaus A, Park WC, Prabhasawat P, John T, McLeod SD, Steuhl KP, Tseng SC. (2000) Conducted a study to determine whether preserved human amniotic membrane (AM) can be used to treat ocular burns in the acute stage. They concluded that amniotic membrane transplantation is effective in promoting re-epithelialization and reducing inflammation, thus preventing scarring sequelae in the late stage. In mild to moderate burns, AMT alone rapidly restores both corneal and conjunctival surfaces. In severe burns, however, it restores the conjunctival ocular surface without debilitating symblepharon and reduces limbal stromal inflammation, but does not prevent limbal stem cell deficiency, which requires further limbal stem cell transplantation.

Honavar SG, Bansal AK, Sangwan VS, Rao GN.(2000) Conducted a study to evaluate amniotic membrane transplantation (AMT) for ocular surface reconstruction in Stevens-Johnson syndrome (SJS).Ten

consecutive patients (10 eyes) with SJS that underwent AMT as the first step in staged ocular surface reconstruction were included. Symblepharon release, excision of epibulbar fibrous tissue, and clearing of the fibrovascular membrane over the cornea was performed in all cases. Amniotic membrane covered the entire bulbar surface up to the fornices in five eyes; cornea and the perilimbal area in two eyes; cornea, the inferior bulbar surface, and the lower fornix in two eyes; and cornea and the superior bulbar surface in one eye. Obliterated fornices were deepened by use of fornix-formation sutures in all eyes. Symblepharon ring was placed postoperatively for 3 weeks to 2 months..Complete corneal reepithelization occurred in all eyes between 1 and 6 weeks. Adequate bulbar surface and fornix depth were achieved in nine eyes, all of which were free of symblepharon at the final follow-up visit. Cicatricial entropion resolved in four of five lower eyelids and one of two upper eyelids after AMT. One patient had a central corneal melt that required or necessitated a penetrating keratoplasty. They concluded that AMT restores adequate bulbar surface and fornix depth and prevents recurrence of symblepharon in severe cases of SJS.

Hong-Jeng Chen, Renato T F Pires, Scheffer C G Tseng (2000)

Conducted a study to evaluate whether amniotic membrane transplantation can be an effective alternative treatment for neurotrophic

corneal ulcers. Amniotic membrane transplantation was performed in 16 eyes of 15 patients with neurotrophic corneal ulcers and vision equal to or worse than 20/200. All but four (76.4%) instances of amniotic membrane transplantation achieved rapid epithelialisation in 16.6 (9.0) days. Of the four eyes showing delayed healing, three eyes healed by tarsorrhaphy, and the remaining one eye with corneal perforation required penetrating keratoplasty and tarsorrhaphy. Two eyes gained vision better than 20/200. The healed corneal surface was accompanied by reduced inflammation. They concluded that amniotic membrane transplantation can be considered an effective alternative for treating severe neurotrophic corneal ulcers.

Meller D, Maskin SL, Pires RT, Tseng SC.(2000) Conducted a study to determine whether preserved human amniotic membrane can restore the large conjunctival defect created during surgical removal of conjunctivochalasis. Amniotic membrane transplantation was performed at two facilities in 40 consecutive patients (47 eyes) with symptomatic conjunctivochalasis refractory to conventional treatments.Epithelial defects healed in 16.5 +/- 7.3 days. They concluded that amniotic membrane transplantation can be considered as an effective means for conjunctival surface reconstruction during removal of conjunctivochalasis.

Hanada K, Shimazaki J, Shimmura S, Tsubota K.(2001) Conducted a study to examine the efficacy of amniotic membrane transplantation in the treatment of deep corneal and scleral ulcers.A total of 11 patients were recruited for this study: four patients (four eyes) with corneal perforation, five patients (five eyes) with a deep corneal ulcer and descemetocele, and two patients (two eyes) with a scleral ulcer. Ulcers were treated by amniotic membrane transplantation. Separate amniotic membranes were transplanted as material to fill the stromal layer (amniotic membrane filling), as a basement membrane (amniotic membrane graft), and as a wound cover (amniotic membrane patch). Eight eyes (72.7%) healed with epithelialization in 16.5 +/- 8.0 days (range, 7 to 29 days), with five and three eyes showing corneal epithelialization and conjunctival epithelialization, respectively. They concluded that multilayered amniotic membrane transplantation may be effective for the treatment of deep ulceration of the cornea and sclera. In some eyes with total corneal limbal dysfunction or autoimmune disorders, amniotic membrane transplantation alone is not effective.

Prabhasawat P, Kosrirukvongs P, Booranapong W, Vajaradul Y.(2001) Studied the efficacy of amniotic membrane transplantation in various indications for ocular surface reconstruction. Amniotic membrane transplantations were performed in 140 eyes (130 patients) for ocular

surface reconstruction. The indications for the corneal group were limbal stem cell deficiency, bullous keratopathy, persistent epithelial defect, band keratopathy, prosthesis, corneal ulcer and acute chemical burn. The indications for the conjunctival group were grafts for pterygium, conjunctival tumors, symblepharon, and covering the scleral graft. Success was noted in 75.7 per cent (106/140) eyes, partial success in 17.9 percent (25/140) eyes, and failure in 6.4 per cent (9/140) eyes for a mean follow-up of 6.6 months (1-19 months). The success and partial success rate were 80.6 per cent (54/67), 14.9 per cent (10/67) in the corneal group and 71.2 per cent (52/73), 20.6 per cent (15/73) in the conjunctival group. They concluded that amniotic membrane transplantation can solve some difficult ocular surface problems, and can be used to promote epithelial healing, reduce inflammation and scarring.

Dekaris I, Gabric N, Mravicic I, Karaman Z, Katusic J, Lazic R, Spoljaric N. (2001) Conducted a study to evaluate the efficacy of multilayer amniotic transplantation (AMT) for reconstruction of corneal stroma and epithelium. Corneal ulcer (28) was a consequence of a previous infectious or neurotrophic keratitis. In the first group (17) ulcer was covered with monolayer AM, while in the other group (11) there were two or more layers of AM situated in the ulcer and the whole cornea was covered with AM sheet. Monolayer AMT was successful in 64%

while the multilayer AMT success rate was 72%. AM gradually dissolved within 3-6 postoperative weeks. They concluded that AM transplantation facilitates rapid healing of corneal epithelium, reduces inflammation and stimulates epithelial cell regrowth. In eyes with deep corneal ulcer multilayer technique proved to be better then monolayer procedure.

Duchesne B, Tahi H, Galand A. . (2001) Repaired corneal perforation using human fibrin glue (HFG) and amniotic membrane transplant (AMT). Three patients in whom central corneal perforations, approximately 2 mm in diameter, occurred after ocular or systemic disease were successfully cured using HFG and AMT.After a follow-up period of 195-325 days, all corneas remained stable; there was no infection or ulcer recurrence, but some corneal scar thinning was observed in all three cases. The described surgical approach using HFG and AMT allowed a successful repair of corneal perforations with a diameter of 2 mm associated with significant loss of stroma. They concluded that this method may be a good alternative to delay penetrating keratoplasty for treating corneal perforations, especially in acute cases in which graft rejection risk is high.

Anderson DF, Ellies P, Pires RT, Tseng SC (2001) conducted a study to examine the efficacy, safety, and long term outcomes of amniotic membrane transplantation for corneal surface reconstruction in cases of

partial limbal stem cell deficiency.17 eyes of 15 patients with partial limbal stem cell deficiency underwent superficial keratectomy of the conjunctivalised corneal surface followed by amniotic membrane transplantation. Cases were followed up for at least a year. All eyes exhibited a stable, intact corneal epithelial surface after a mean follow up period of 25.8 months with no eyes developing recurrent erosion or persistent epithelial defect. Overall improvement in visual acuity was observed in 92.9% of 14 eyes with visual potential Pain and photophobia were abolished in 86% of cases and substantially reduced in 14%, with all eyes exhibiting decreased vascularisation and inflammation at final follow up. They concluded that amniotic membrane transplantation appears to be a safe and effective method of restoring a stable corneal epithelium for cases of partial limbal stem cell deficiency and can be considered as an alternative to limbal autograft or allograft.

Letko E, Stechschulte SU, Kenyon KR, Sadeq N, Romero TR, Samson CM, Nguyen QD, Harper SL, Primack JD, Azar DT, Gruterich M, Dohlman CH, Baltatzis S, Foster CS (2001) Conducted a study to determine the effect of amniotic membrane transplantation (AMT) on persistent corneal epithelial defects (PEDs) and to compare the efficacy between inlay and overlay techniques. Thirty patients (30 eyes) underwent AMT for PED. The amniotic membrane was placed on the

surface of the cornea in overlay (group A) or inlay (group B) fashion.The PED healed after the first AMT in 21 eyes (70%) within an average of 25.5 days after surgery and recurred in 6 eyes (29%). Among the 22 eyes treated with an overlay AMT (group A), the PED healed after the first AMT in 14 eyes (64%) within an average of 24.5 days and recurred in 4 eyes (29%). Among the 8 eyes treated with an inlay AMT (group B), the PED healed within an average of 27.4 days after AMT, which did not statistically significantly differ from group A (P = .72). The PED healed after the first AMT in 7 eyes (88%) and recurred in 2 (29%) of 7 eyes. They concluded that the AMT can be helpful in the treatment of PED in which all other conventional management has failed. They did not find any difference between overlay and inlay techniques in terms of healing time and recurrence rate.

Pinnita Prabhasawat, Nattaporn Tesavibul, Wiwat Komolsuradej (2001) Conducted a study to evaluate the efficacy of amniotic membrane transplantation (AMT) in persistent corneal epithelial defect with or without stromal thinning and corneal perforation.28 patients (28 eyes) with persistent corneal epithelial defect unresponsive to medical treatment were given preserved human amniotic membrane transplants. The patients were divided into three groups: group A, persistent corneal epithelial defect 10 eyes; group B, epithelial defect with

stromal thinning 13 eyes; and group C, corneal perforation five eyes. Result is summarized in the following table.

Indication	No. of cases	Success rate
Persistent corneal epithelial defect	10	80%
Persistent corneal epithelial defect with stromal thinning	13	84.6%
Corneal perforation	5	80%

They concluded that amniotic membrane can successfully treat refractory corneal epithelial defect by promoting epithelial healing and thus prevent corneal perforation. It can be used as a treatment for corneal perforation by restoring corneal stromal thickness so that emergency penetrating keratoplasty can be avoided.

Mrukwa-Kominek E, Gierek-Ciaciura S, Rokita-Wala I, Szymkowiak M (2002) The study aimed to evaluate effectiveness of bullous keratopathy treatment using amniotic membrane transplantation, as an alternative method of treatment after cataract surgery. The amniotic membrane transplantation was performed in 18 eyes of 18 patients.After removing the pathologically changed epithelium, the amniotic membrane

was covered on cornea in local anesthesia, using interrupted sutures 10.0 nylon. In all the patients' regression of subjective complaints was observed (pain, light sensitivity and tearing). In 12 cases improvement of visual acuity was achieved.

They concluded that the amniotic membrane transplantation is an effective method of treatment for bullous keratopathy and has beneficial influence on the process of corneal healing and the improvement of visual acuity and diminish subjective symptoms.

Solomon A, Meller D, Prabhasawat P, John T, Espana EM, Steuhl KP, Tseng SC (2002) Described the clinical outcome of amniotic membrane transplantation (AMT) for nontraumatic corneal perforations, descemetoceles, and deep ulcers. Thirty-four eyes of 33 consecutive patients operated on for nontraumatic corneal perforations or descemetoceles. Three or four layers of amniotic membrane (AM) were applied over the ulcer bed and anchored with 10-0 nylon interrupted or running sutures. A large AM piece was used as a patch to cover the entire corneal surface. Formation of anterior chamber depth, epithelialization of the AM grafts, and stability of the corneal stromal thickness. A successful result was observed in 28 of 34 eyes (82.3%). They concluded that AMT is an effective method for managing nontraumatic corneal perforations and descemetoceles. It can serve as either a permanent therapy or as a

temporizing measure until the inflammation has subsided and a definitive reconstructive procedure can be performed.

Pan Z, Zhang W, Wu Y, Sun B (2002) conducted a study to investigate the proliferation and differentiation of cultured corneal stem cells and determine the effect of corneal stem cells cultured on amniotic membranes on the limbal area for treating corneal burns. The stem cells could proliferate to form cell layer on an amniotic membrane. When transplanted, stem cells could survive on limbus. After transplantation, ocular inflammation resolved, the cornea re-epithelialized, the stromal opacity reduced, the superficial neovascularity was lessened and the conjunctival fornix re-established. They concluded that ocular surface conditions could be improved by allograft of corneal stem cells cultured on amniotic membranes.

Zito E, Borderie V, Touzeau O, Bourcier T, Allouch C, Laroche L (2002) Conducted a study to evaluate amniotic membrane transplantation (AMT) in severe corneal epithelial diseases. Amniotic membrane transplantation was performed in 14 eyes of 14 patients. Patients with corneal stable reepithelialization, no corneal neovascularization, and no recurrence of the initial pathology were considered successful. All but three patients underwent corneal reepithelialization within 6 weeks of AMT, with a mean healing time of

31+/-23 days. The success rate was 75% at 6 months. They concluded that AMT is a useful technique for ocular surface reconstruction, especially in association with limbal transplantation. It could also improve the prognosis of penetrating keratoplasty in patients with severe corneal conditions.

Solomon A, Espana EM, Tseng SC (2003) conducted a study to describe the clinical outcome of amniotic membrane transplantation (AMT) for fornix reconstruction in a variety of ocular surface disorders.. Four eyes had ocular-cicatricial pemphigoid, two eyes had symblepharon after pterygium excision, four eyes had chemical or mechanical trauma, two eyes had strabismus surgery, two eyes (one patient) had Stevens-Johnson syndrome, one eye had toxic epidermal necrolysis, and two eyes (one patient) had chronic allergic conjunctivitis.The subconjunctival scar tissue was dissected from the episclera, and the freed conjunctival flap was recessed to the fornix. A layer of amniotic membrane (AM) was applied to cover the exposed episclera. The fornical edge of the membrane was anchored with sutures passing through the full thickness of the lid. A deep conjunctival fornix, lack of motility restriction. Complete fornix reconstruction was demonstrated in 12 of 17 eyes (70.6%), whereas 2 eyes had a partial success, and 3 eyes (3 patients) had recurrence of symblepharon with restricted motility. The most successful

outcome was observed in eyes with symblepharon associated with trauma. They concluded that AMT is an effective method of fornix reconstruction for the repair of symblepharon in a variety of ocular surface disorders.

Barabino S, Rolando M, Bentivoglio G, Mingari C, Zanardi S, Bellomo R, Calabria G. (2003) Conducted a study to evaluate the role and the effectiveness over time of amniotic membrane transplantation (AMT) as a first-step procedure to treat conjunctival reconstruction in late-stage ocular-cicatricial pemphigoid (OCP Nine eyes (9 patients) with advanced OCP. After scar tissue was removed, the preserved amniotic membrane was placed over the cornea, the bulbar, and tarsal conjunctiva, and was secured with 8-0 Vicryl sutures to the conjunctival edges and the deep fornices with double-armed 6-0 silk sutures. The conjunctival surface was free from symblepharon in all subjects for the first 16 weeks.. They concluded that AMT can be a first-step procedure for ocular surface reconstruction in OCP, but its effectiveness deteriorates slightly over time.

Meallet MA, Espana EM, Grueterich M, Ti SE, Goto E, Tseng SC. (2003) Conducted a study to evaluate the outcomes of corneal surface reconstruction with conjunctival limbal autograft when combined with amniotic membrane transplantation on both the donor and recipient

eyes. Five eyes of five patients with total limbal stem cell deficiency (LSCD) resulting from pseudopemphigoid (n = 1), chemical burns (n = 3), and extensive removal of conjunctival intraepithelial neoplasia (n = 1) were operated on by one surgeon (SCGT).After the removal of fibrovascular pannus from the corneal surface, two conjunctival limbal free grafts were harvested from the fellow eyes in all five patients with unilateral LSCD. Amniotic membrane, with the basement membrane side up, was grafted onto the defect created at the donor site and onto the recipient corneal and limbal sclera before placement of conjunctival limbal grafts.Symptomatic relief, improvement in visual acuity, fornix deepening, and rapid healing and restoration of normal cornea and limbus in the recipient and donor eyes were assessed.all eyes experienced symptomatic relief. All recipient eyes had a mean improvement in visual acuity of nine lines (range, 7-12). The three eyes with stromal vascularization showed regression, and all recipient eyes had marked improvement in corneal clarity. Three eyes receiving simultaneous symblepharon lysis and fornix reconstruction successfully regained deep, stable fornices. The donor eyes showed rapid healing and restoration of the normal limbal landmark, even in one eye where nearly the entire limbus was removed. They concluded that limbal conjunctival

transplantation is an effective procedure for restoring the corneal surface integrity in eyes with total LSCD.

AIMS AND OBJECTIVES

Aims & Objectives

The present clinical study was undertaken to review the characteristics of amniotic membrane that make it potentially useful to treat ocular surface abnormalities and to discuss the current indications, the surgical technique, and the outcome of AMT. The aims and objectives of the study were: -

1. To determine the efficacy of AMT in its healing effect in various ocular surface disorders such as nonhealing corneal ulcer, persistent epithelial defect and descemetocele.
2. To determine the improvement in visual acuity after healing of corneal ulcer due to less scarring.
3. To assess the efficacy of AMT in reducing pain caused by corneal pathologies,such as bullous keratopathy.
4. To study the efficacy of amniotic membrane graft after removal of pterygium.
5. To observe how it reduces the adhesion of tissue in case of symblepharon correction.

MATERIAL AND METHODS

Material & Methods

The present prospective study was carried out at department of ophthalmology, M.L.B. Medical college, Jhansi.48 patients with either nonhealing corneal ulcer, persistent epithelial defect, descemetocele, bullous keratopathy, pterygium and symblepharon were selected from those attending the eye OPD between June 2001 to Aug 2003. An informed consent patient data including the demographic factors, previous medical, surgical and ocular history, were recorded. Clinical data, indications for surgeries and goal of treatments in each group are summarized in Table- A.

TABLE A

INDICATIONS FOR SURGERY AND CRITERIA FOR SUCCESS, PARTIAL SUCCESS AND FAILURE IN EACH CATEGORY

Indication for surgery	**Success**	**Partial success**	**Failure**
Non healing corneal ulcer	Healed ulcer	Incomplete healing	Non healing
Persistent epithelial defect	Healed epithelial defect	Incomplete healing	Non healing
Descemetocele	Complete healing	Incomplete healing	Non healing
Bullous keratopathy	Relieved pain and irritation	Transient relief in pain and irritation	Persistence of pain and irritation
Symblepharon	Released symblepharon	Some fibrovascular tissue	Recurrence

PREOPERATIVE EVALUATION

All the cases were examined for best corrected visual acuity. Slit lamp examination was done for tear film evaluation and measurement of epithelial defect. Lid was examined to rule out entropion and trichiasis and meibomitis, which were treated before AMT. topical antbiotic and artificial tear drops 4 times given at least 1 week before surgery.

PREPARATION OF AMNIOTIC MEMBRANE

Fresh Amniotic membrane was harvested from consenting seronegative (hepatitis B and C virus, syphilis and human immunodeficiencv virus) maternal donors during elective caesarian section. Under sterile conditions, the placental membrane was washed in a balanced salt solution (BSS) to remove clots and debris. The membrane was then bathed in a cocktail of antimicrobial medium and stored at 4°C. The membrane was used in 24 hr of harvest.

SURGICAL TECHNIQUE

At the time of surgery amnion was seperated from chorion.After retrobulbar anaesthetic injection in eyes with deep corneal ulcer, the base of the ulcer was debrided, and the poorly adherent epithelium adjacent to the edge of the ulcer was removed up to the area where the epithelium became adherent. The amniotic membrane was transferred to the recipient

eye, and fitted to fill up the ulcer and cover the defect by trimming off the excess edges. This fashioned membrane with stroma-side down was then secured to the edge of the defect by interrupted 10-0 nylon sutures.

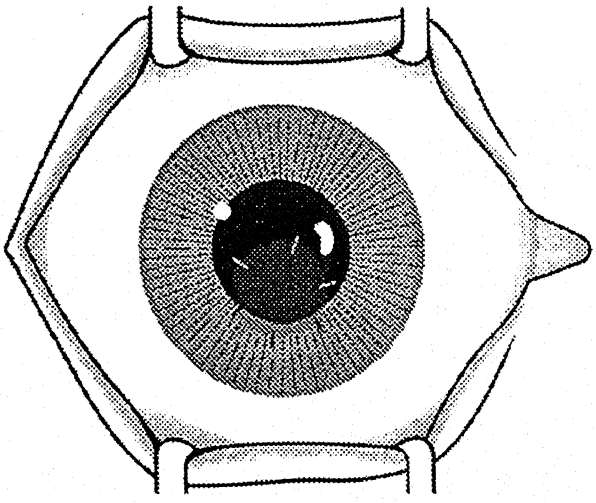


Fig-1 :

The diagram illustrates the amniotic membrane sutured to the cornea and covering a paracentral corneal epithelial defect.

Alternatively, in case of a large ulcer, the AM was sutured to perilimbal episclera and the edge of the conjunctiva after peritomy covering the whole corneal surface.

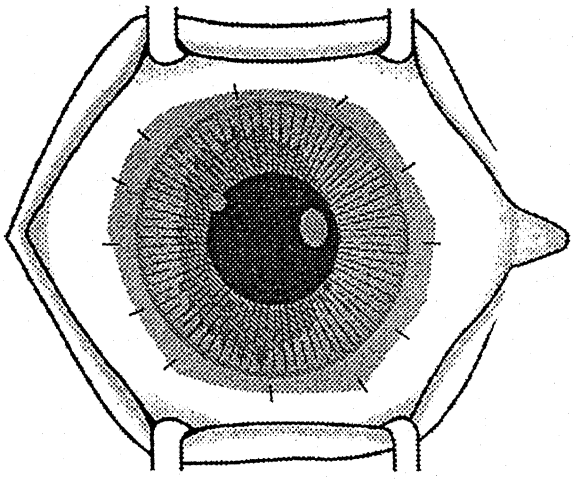


Fig-2

The amniotic membrane is sutured to perilimbal episclera and to the edge of the conjunctiva (after peritomy) covering the whole corneal surface.

After the knots had been buried, the corneal surface became smooth as a result of the well-approximated amniotic membrane filling in the ulcer bed. Single layer amniotic membrane was used for persistent epithelial defect and Bullous keratopathy, More than one layer of amniotic membrane was used if the ulcer was deep and in those instances the bottom layers were left unsutured and only the top layer was sutured. Multilayer amniotic membrane was also applied in cases with descemetocele and in cases with symblepharon.

PRINCIPLE OF MULTILAYER AMNIOTIC MEMBRANE TRANSPLANTATION

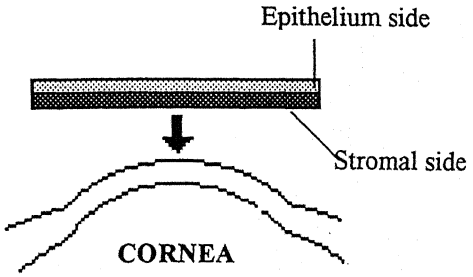


Fig- 3
Amniotic membrane as a graft with epithelial surface facing upward

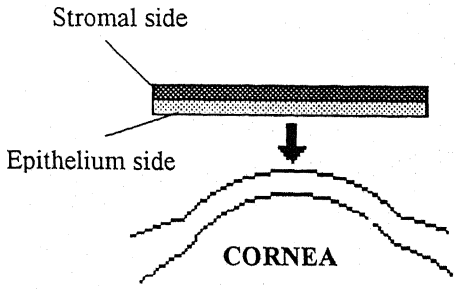


Fig –4
Amniotic membrane as a patch with epithelial surface facing down

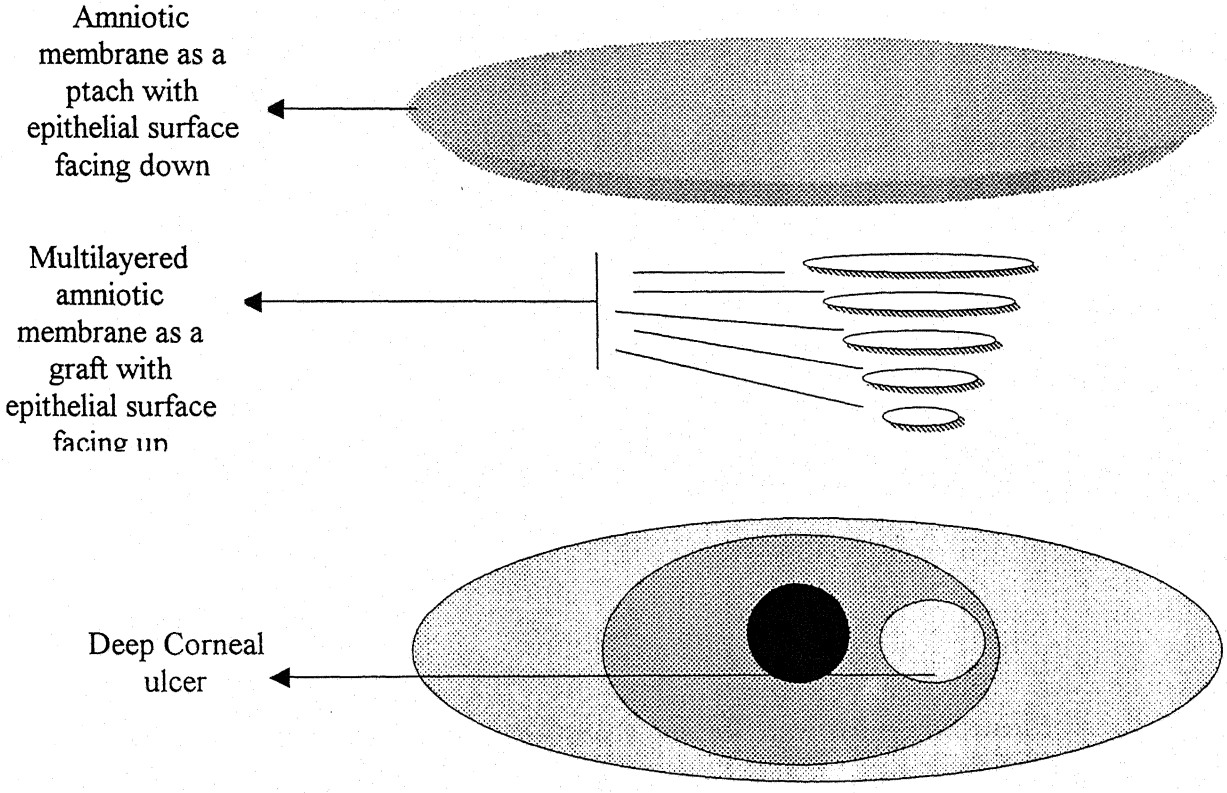


Fig –5

Multilayer amniotic membrane

Depending on the aqueous tear status and the eyelid blinking function, amniotic membrane as a temporary patch, or temporary tarsorrhaphy was added. **When amniotic membrane was used as a patch**, this was performed by placing an amniotic membrane over the cornea or extending it beyond the limbus with the basement membrane side facing down, and was sutured with interrupted 10-0 nylon sutures. In pterygium or symblepharon surgery, the membrane is applied to cover areas of conjunctival defects after removal of fibrotic tissue. The membrane was secured to the surrounding conjunctival edge with episcleral bites and at the lid margins using interrupted 9-0 or 10-0 Vicryl sutures .To ensure the depth of the newly created lower and upper fornices, the AM was secured with two double-armed horizontal mattress sutures of 6-0 silk, which were brought temporally and nasally through the lid and tied over the skin with bolsters.

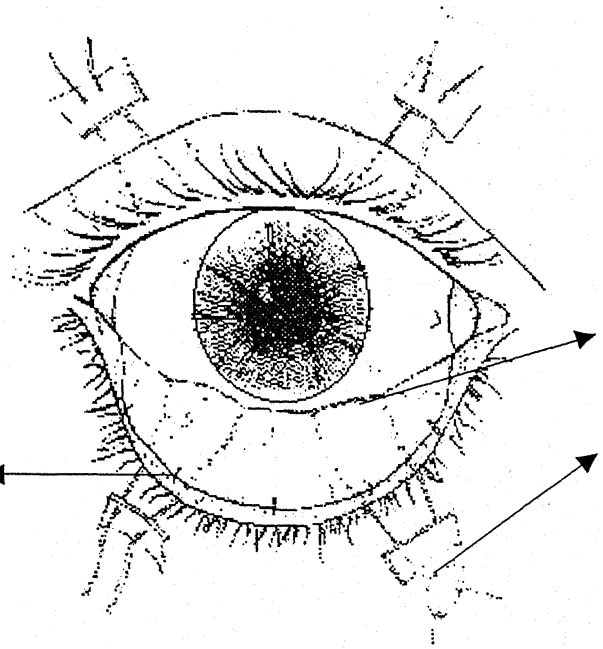


Fig –6

Method of AMT for symblepharon

POSTOPERATIVE CARE AND EVALUATION

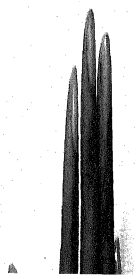
Before epithelialisation the patient was followed weekly and was routinely treated with topical 1% prednisolone acetate, three times a day and 0.3% ofloxacin twice a day. After epithelialisation was completed, the latter was discontinued but the former was tapered off. Fluorescein staining was used to detect epithelial defects. Following healing, topical antibiotics were discontinued and the topical steroid was tapered off.

COMLICATIONS

Immediate complications, i.e., those developing within 1 month of surgery, detachment of amniotic membrane and conjunctivitis, lid abscess. Despite of detachment of amniotic membrane, the epithelial defect healed rapidly in 1 week in the patient with bullous keratopathy so that the patient became pain-free. The eye, which developed conjunctivitis was from a patient with Steven-Johnson syndrome as a result of bacterial infection and the membrane was detached and dissolved in that area. This complication was successfully treated with antibiotics .

Late complications was those which developed after 1 month of surgery and the epithelial surface had healed completely. It was corneal ulcer.

OBSERVATIONS



Observations

The present prospective study *"Amniotic membrane transplantation for ocular surface disorders"* was carried out in the department of Ophthalmology, MLB Medical College, Jhansi. Forty eight patients with either nonhealing corneal ulcer, persistent epithelial defect, descemetocele, bullous keratopathy, pterygium and symblepharon were studied, all of which were operated by the above said procedure. Postoperatively the follow up period ranged from 3-10 months with a mean of 10 months.

The various ocular surface disorders for which AMT was done are shown in table-1. The maximum number of cases were those of non-healing corneal ulcer.

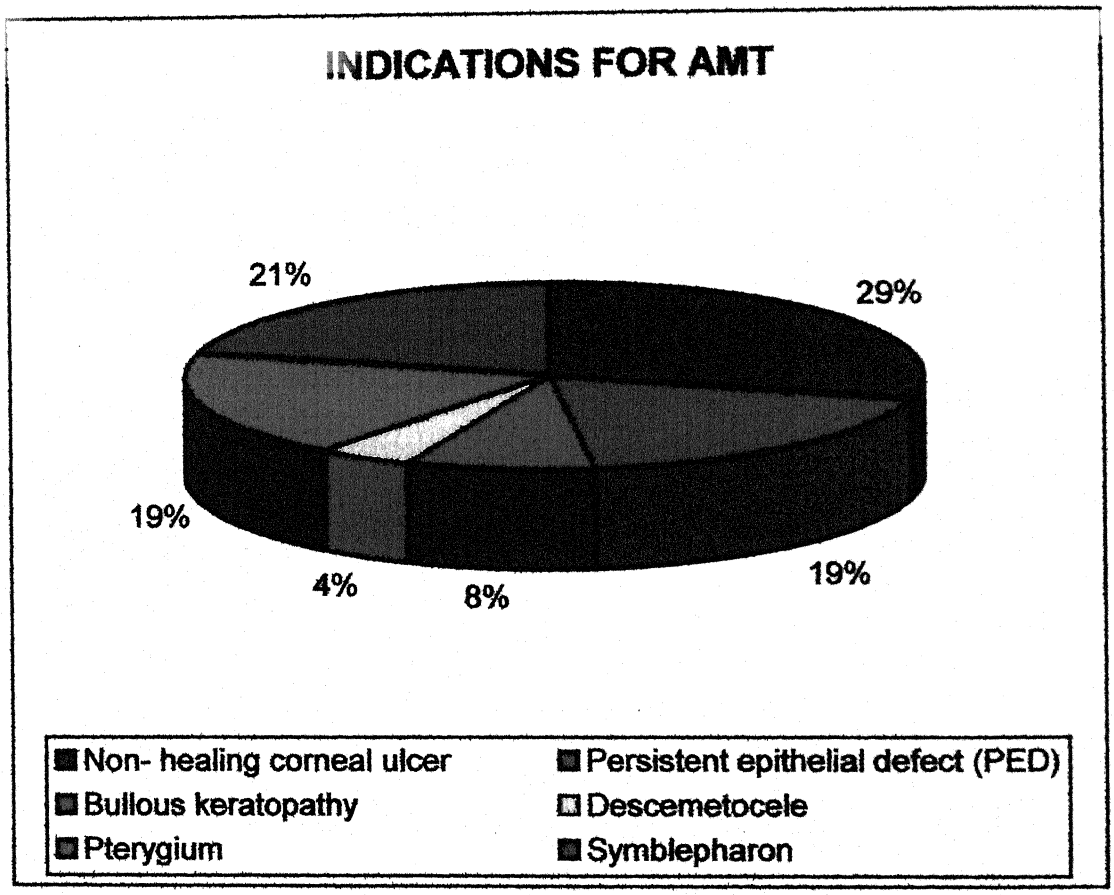

INDICATIONS FOR AMT
21%
29%
19%
4%
8%
19%
Non- healing corneal ulcer
Persistent epithelial defect (PED)
Bullous keratopathy
Descemetocele
Pterygium
Symblepharon

TABLE-1

INDICATIONS FOR AMT

Indication	No. of cases	Percentage
Non- healing corneal ulcer	14	29.16%
Persistent epithelial defect (PED)	09	18.75%
Descemetocele	02	4.44%
Bullous keratopathy	04	8.33%
Pterygium	09	18.75%
Symblepharon	10	22.22%
Total	**48**	**100%**

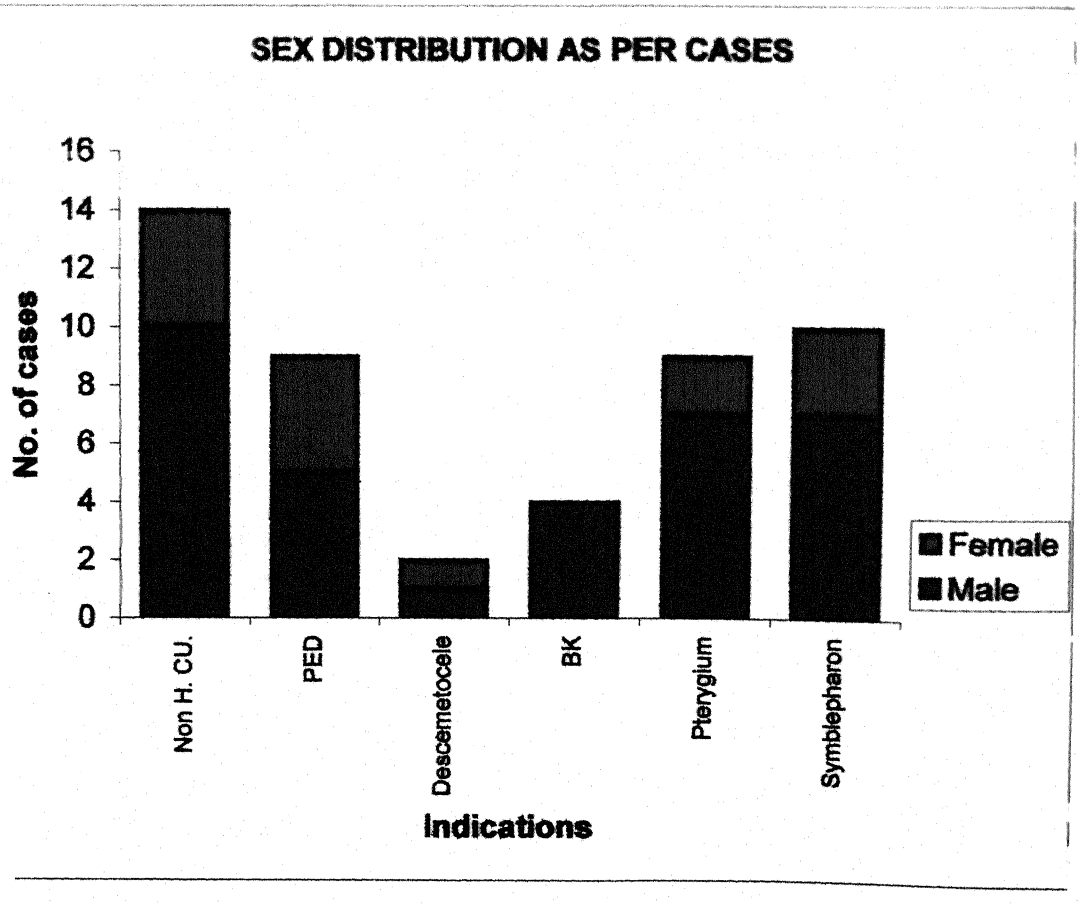

SEX DISTRIBUTION AS PER CASES
No. of cases
16
14
12
10
8
6
4
2
0
Non H. CU.
PED
Descemetocele
BK
Pterygium
Symblepharon
Indications
Female
Male

TABLE-2

SEX DISTRIBUTION AS PER CASES

Sex	Indications						Total	%age
	Non H. CU.	PED	Descem etocele	BK	Pterygium	Symblepharon		
Male	10	05	01	04	07	07	34	70.83
Female	04	04	01	00	02	03	14	29.16
Total	**14**	**09**	**02**	**04**	**09**	**10**	**48**	**100**

There was a predilection among males due to a predominantly outdoor nature of their work and higher exposure to radiation, trauma and infection. Among the females, 70% of these affected were involved in regular outdoor work (mostly agricultural workers).

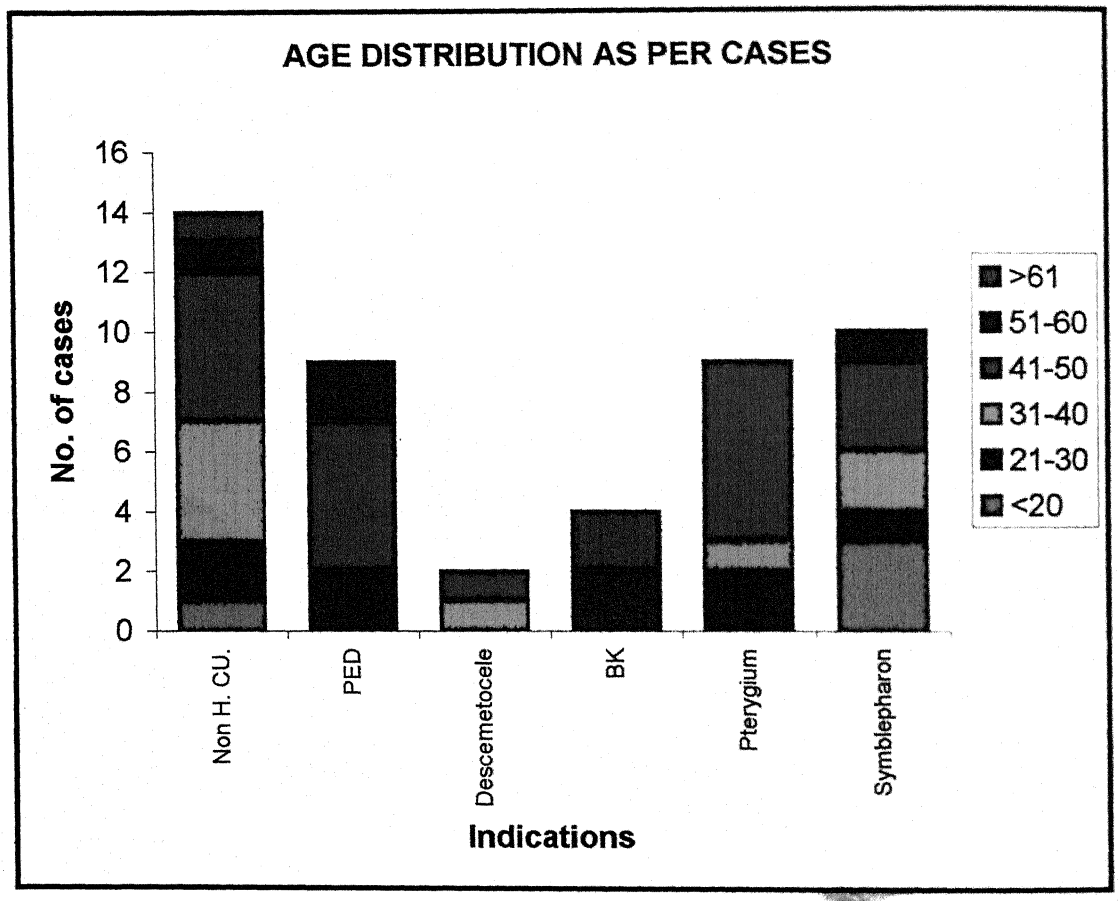

AGE DISTRIBUTION AS PER CASES
No. of cases
16
14
12
10
8
6
4
2
0
Non H. CU.
PED
Descemetocele
BK
Pterygium
Symblepharon
Indications
>61
51-60
41-50
31-40
21-30
<20

TABLE-3

AGE DISTRIBUTION AS PER CASES

Age	Indications						Total	%age
	Non H. CU.	PED	Descemet ocele	BK	Pterygium	Symblepharon		
<20	01	00	00	00	00	03	04	8.33
21-30	02	02	00	00	02	01	07	14.58
31-40	04	00	01	01	01	02	08	16.66
41-50	05	05	01	01	06	03	20	41.66
51-60	01	02	00	02	00	01	06	12.50
>61	01	00	00	02	00	00	03	6.25
Total	**14**	**09**	**02**	**04**	**09**	**10**	**48**	**100**

The age distribution varied over a wide range. The youngest patient in the study was a 12 years old boy with a symblepharon secondary to traumatic injury to the eye while the oldest patient was a 65 years old male with bullous keratopathy. The average age of patients in this study was 46.6 years.

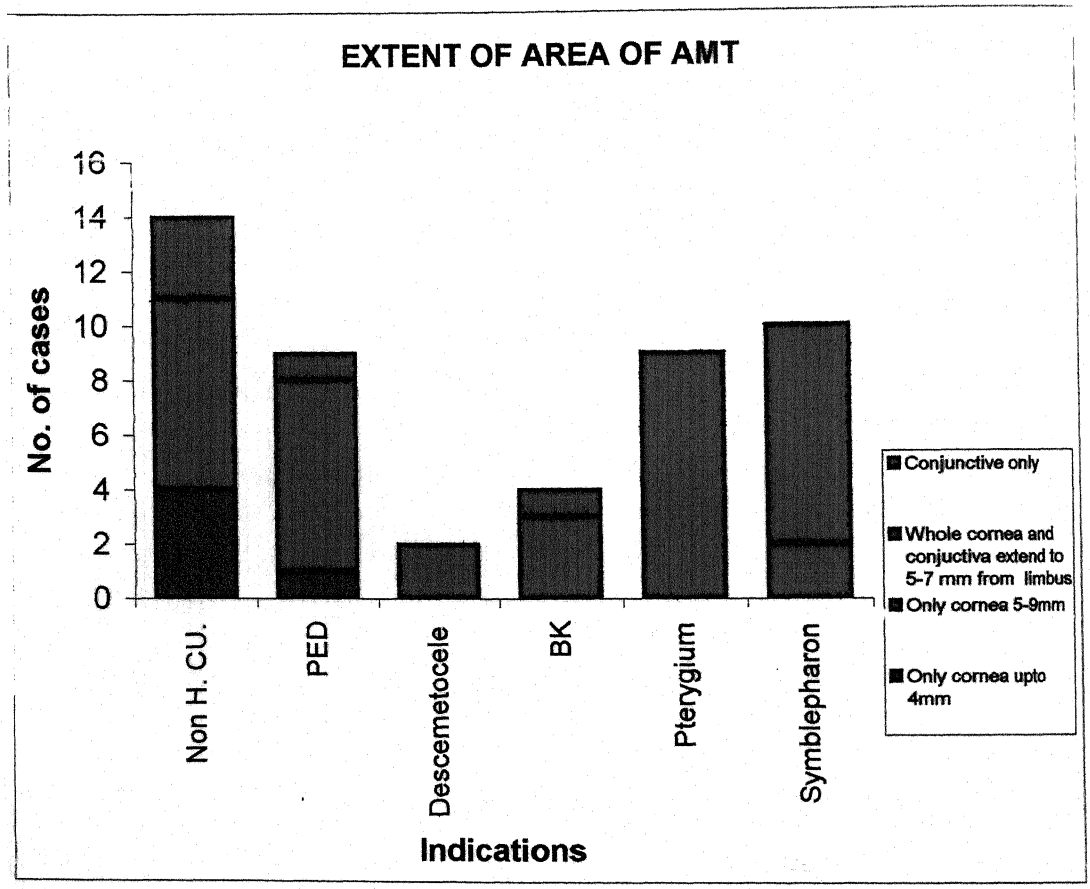

EXTENT OF AREA OF AMT
No. of cases
16
14
12
10
8
6
4
2
0
Non H. CU.
PED
Descemetocele
BK
Pterygium
Symblepharon
Indications
Conjunctive only
Whole cornea and conjuctiva extend to 5-7 mm from limbus
Only cornea 5-9mm
Only cornea upto 4mm

TABLE-4

EXTENT IN AREA OF AMT

Extent	Indications						Total	%age
	Non H. CU.	PED	Descem etocele	BK	Pterygium	Symblepharon		
Only cornea upto 4mm	04	01	00	00	00	00	05	10.41
Only cornea 5-9mm	07	07	02	03	00	00	19	39.58
Whole cornea and conjuctiva extend to 5-7 mm from limbus	03	01	00	01	00	02	07	14.58
Conjunctive only	00	00	00	00	09	08	17	35.41
Total	**14**	**09**	**02**	**04**	**09**	**10**	**48**	**100**

In 04 cases with a localized peripheral non healing corneal ulcer a small graft involving only upto 4mm of the cornea was transplanted. Slightly larger graft of 5-9 mm were needed in larger ulcers, while most of the cases pterygium and symblepharon had only congunctival transplantation of amniotic membrane.

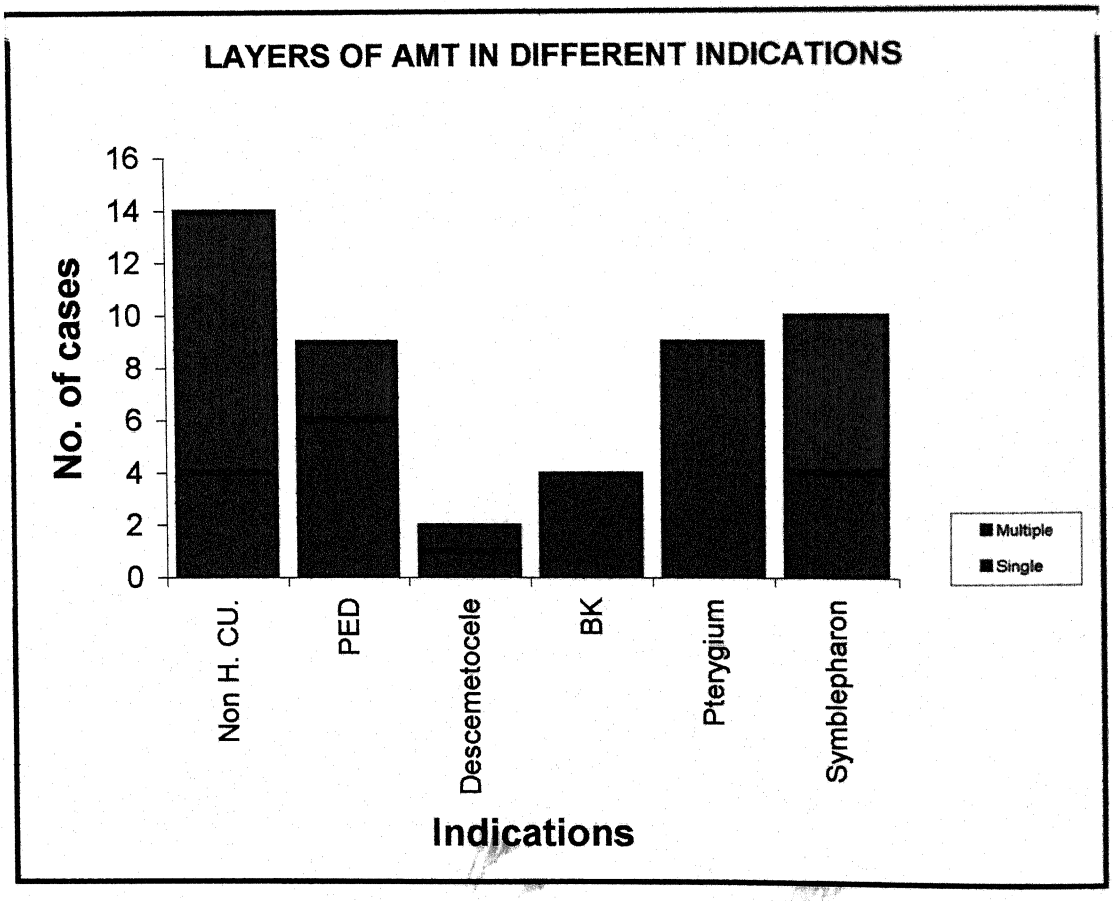

LAYERS OF AMT IN DIFFERENT INDICATIONS
No. of cases
16
14
12
10
8
6
4
2
0
Non H. CU.
PED
Descemetocele
BK
Pterygium
Symblepharon
Indications
Multiple
Single

TABLE-5

LAYERS OF AMT IN DIFFERENT INDICATIONS

No. of layers	Indications						Total	%age
	Non H. CU.	PED	Descem etocele	BK	Pterygium	Symblepharon		
Single	04	06	00	04	09	04	27	58.33
Multiple	10	03	02	00	00	06	21	41.66
Total	**14**	**09**	**02**	**04**	**09**	**10**	**48**	**100**

In 10 out of 14 patients with non healing corneal ulcers, all patients with Descemetocele ,6 patients with Symblepharon multiple layers of AMT was done. In all 4 cases of bullous keratopathy , 9 cases of pterygium and 4 patients with Symblepharon a single layer of AMT was done.

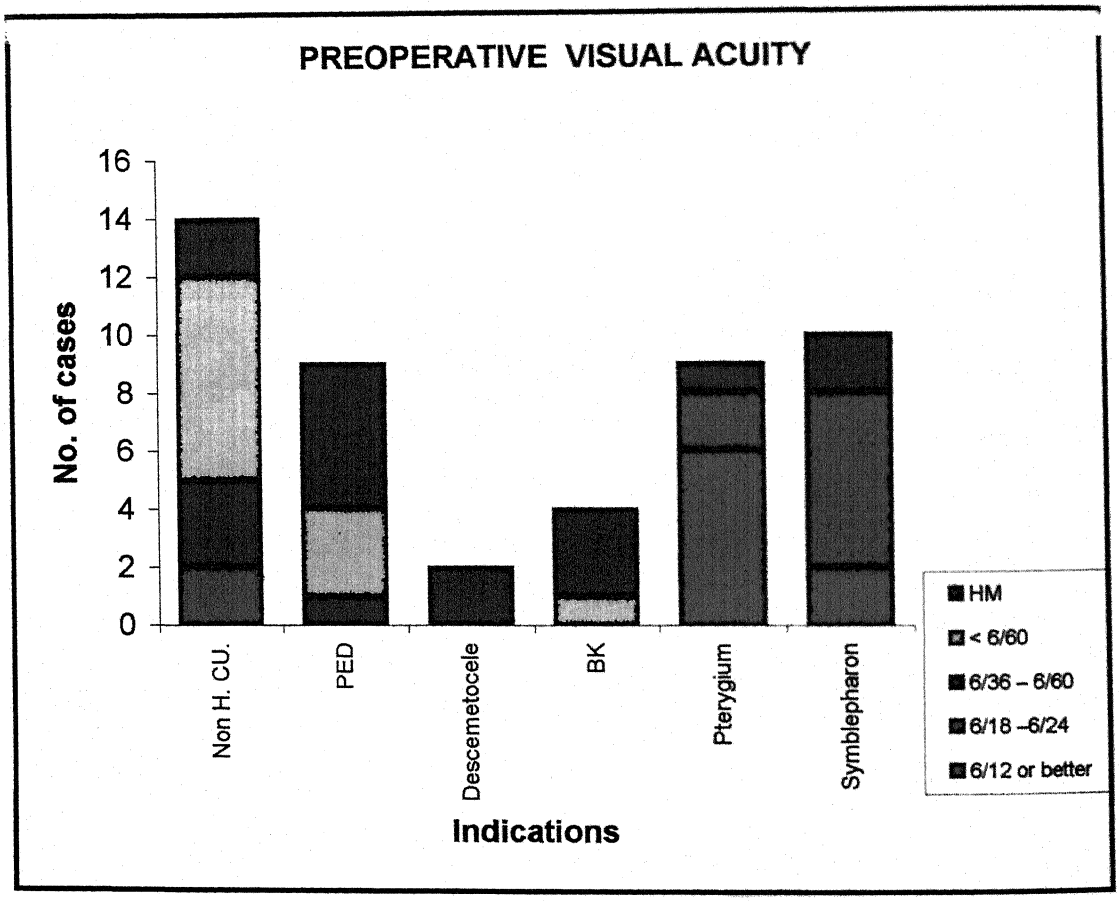

PREOPERATIVE VISUAL ACUITY
No. of cases
16
14
12
10
8
6
4
2
0
Non H. CU.
PED
Descemetocele
BK
Pterygium
Symblepharon
Indications
HM
< 6/60
6/36 – 6/60
6/18 –6/24
6/12 or better

TABLE-6

IMPROVEMENT IN VISUAL ACUITY

PRE-OPERATIVE VISUAL ACUITY

Pre-operative visual acuity	Indications						Total	%age
	Non H. CU.	PED	Descem etocele	BK	Pterygium	Symblepharon		
6/12 or better	00	00	00	00	06	02	08	16.66
6/18 –6/24	02	00	00	00	02	06	10	20.83
6/36 – 6/60	03	01	00	00	01	00	05	10.41
< 6/60	07	03	00	01	00	00	11	22.91
HM	02	05	02	03	00	02	14	29.16
Total	**14**	**09**	**02**	**04**	**09**	**10**	**48**	**100**

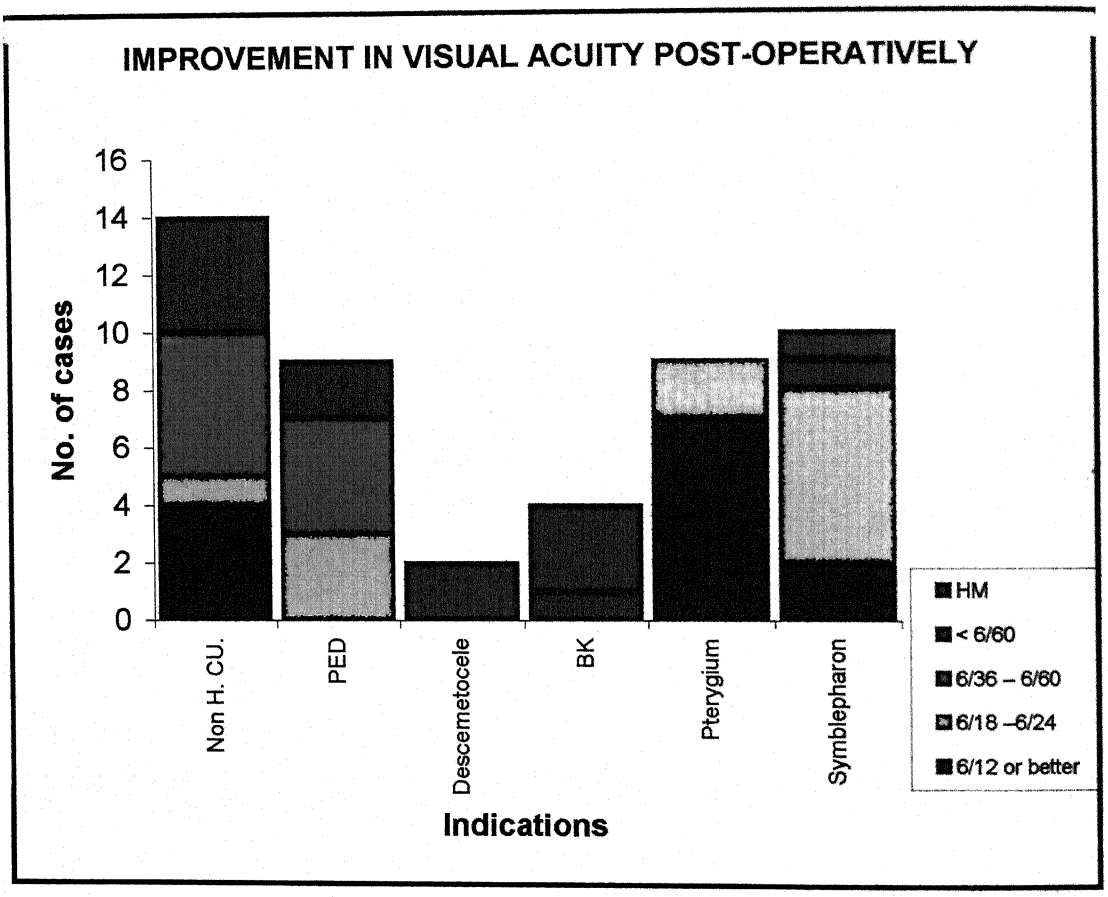

IMPROVEMENT IN VISUAL ACUITY POST-OPERATIVELY
No. of cases
16
14
12
10
8
6
4
2
0
Non H. CU.
PED
Descemetocele
BK
Pterygium
Symblepharon
Indications
HM
< 6/60
6/36 – 6/60
6/18 –6/24
6/12 or better

TABLE-7

IMPROVEMENT IN VISUAL ACUITY

POST OPERATIVE VISUAL ACUITY

Post-operative visual acuity	Indications						Total	%age
	Non H. CU.	PED	Descem etocele	BK	Pterygium	Symblepharon		
6/12 or better	04	00	00	00	07	02	13	27.08
6/18 –6/24	01	03	00	00	02	06	12	25.00
6/36 – 6/60	05	04	02	00	00	00	09	18.75
< 6/60	04	02	00	01	00	01	08	16.66
HM	00	00	00	03	00	01	06	12.50
Total	**14**	**09**	**02**	**04**	**09**	**10**	**48**	**100**

Most of the patients with non healing corneal ulcer and all the case with Descemetocele had an improvement of approximately 2 Sneller's lines after AMT. Similarly patients with PED also had an improved visual acuity after AMT. Although patients with pterygium and symblepharon who had AMT had a slight improvement in visual acuity in one case each, the visual acuity remained the same in case with bullous keratopathy and did not deteriorate further.

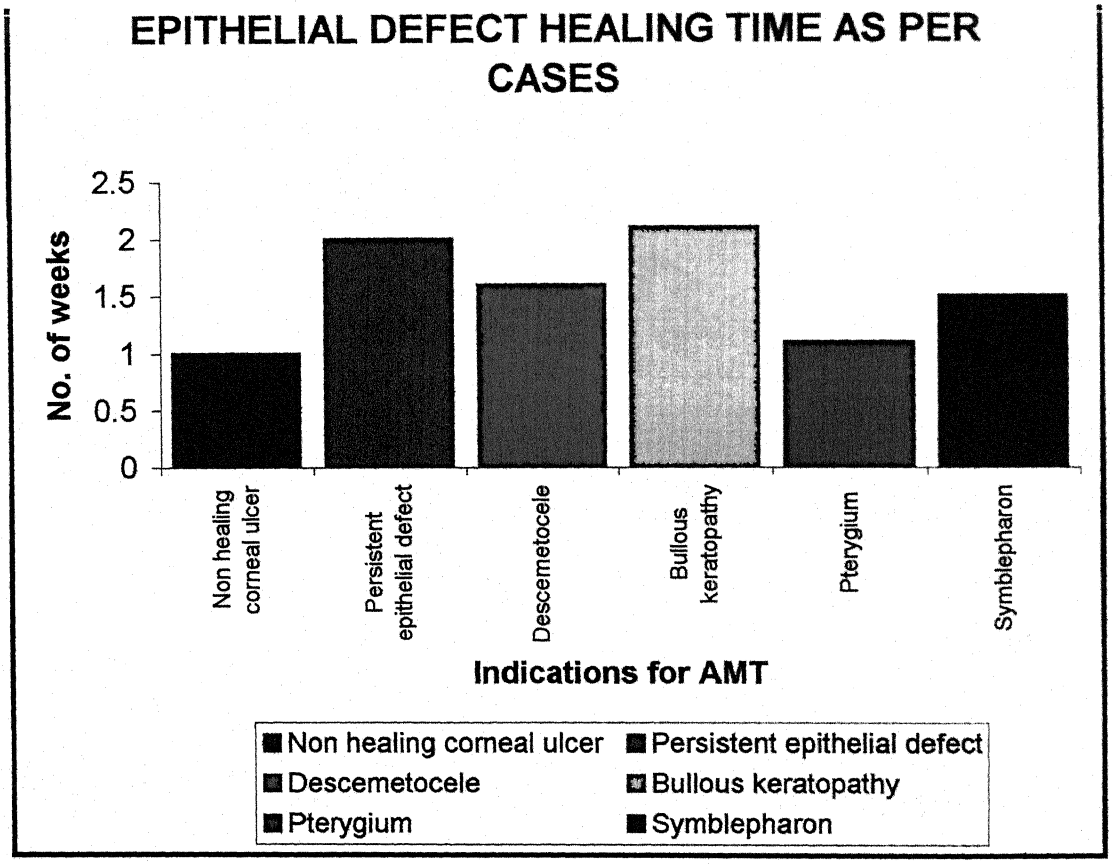
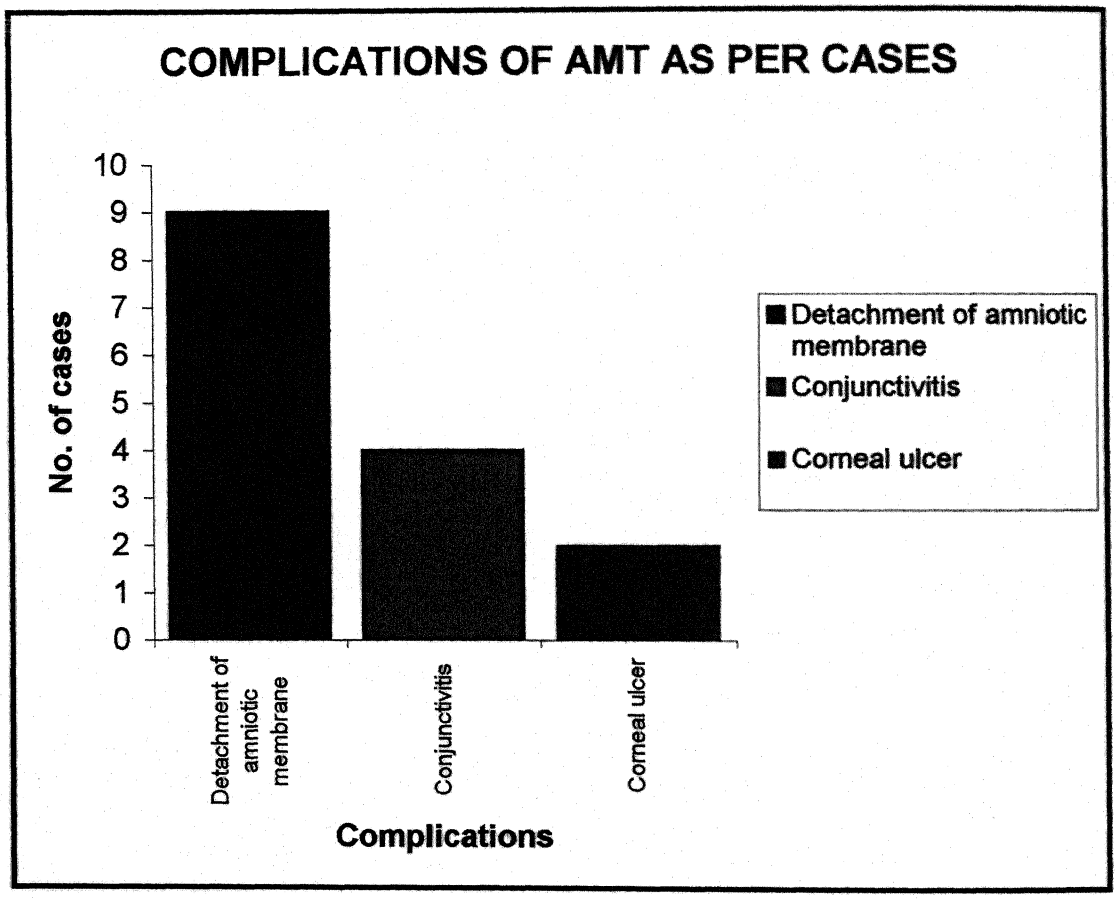

EPITHELIAL DEFECT HEALING TIME AS PER CASES
No. of weeks
2.5
2
1.5
1
0.5
0
Non healing corneal ulcer
Persistent epithelial defect
Descemetocele
Bullous keratopathy
Pterygium
Symblepharon
Indications for AMT
Non healing corneal ulcer
Persistent epithelial defect
Descemetocele
Bullous keratopathy
Pterygium
Symblepharon

TABLE-8

EPITHELIAL DEFECT HEALING TIME AS PER CASES

Indications for AMT	Epithelial defect healing time
Non healing corneal ulcer	1 week
Persistent epithelial defect	2 weeks
Descemetocele	1.6 weeks
Bullous keratopathy	2.1 weeks
Pterygium	1.1 weeks
Symblepheron	1.5 weeks

Non healing corneal ulcers healed rapidly in an average of 1 week time, persistent epithelial defects in 2 weeks, bullous keratopathy in 2.1 weeks respectively while symblepharon cases healed in a average duration of 1.5 weeks.


COMPLICATIONS OF AMT AS PER CASES
No. of cases
10
9
8
7
6
5
4
3
2
1
0
Detachment of amniotic membrane
Conjunctivitis
Corneal ulcer
Complications
Detachment of amniotic membrane
Conjunctivitis
Corneal ulcer

Complications :

The various complications which occurred post operatively have been recorded in the table below :

TABLE-9

COMPLICATIONS OF AMT AS PER CASES

EARLY COMPLICATIONS – developing within 1 month of surgery		
Detachment of amniotic membrane	9 cases	19%
Conjunctivitis	4 cases	8.3%

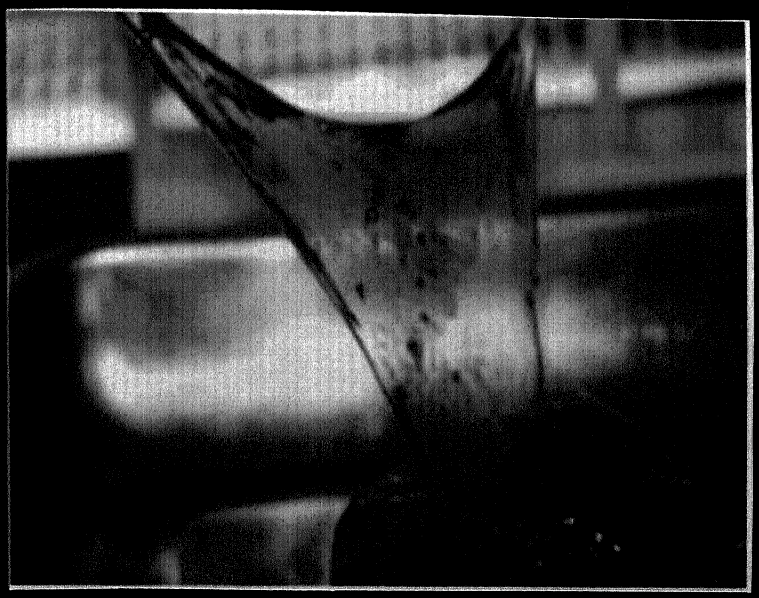
LATE COMPLICATION – developing after 1 month of surgery and after epithelial surface had healed completely		
Corneal ulcer	2 cases	4.1%

RESULTS
No. of cases
12
10
8
6
4
2
0
Non healing corneal ulcer
persistent epithelial defect
Descemetocele
Bullous keratopathy
pterygium
Symblepharon
Indications for surgery
Success
Partial success
Failure

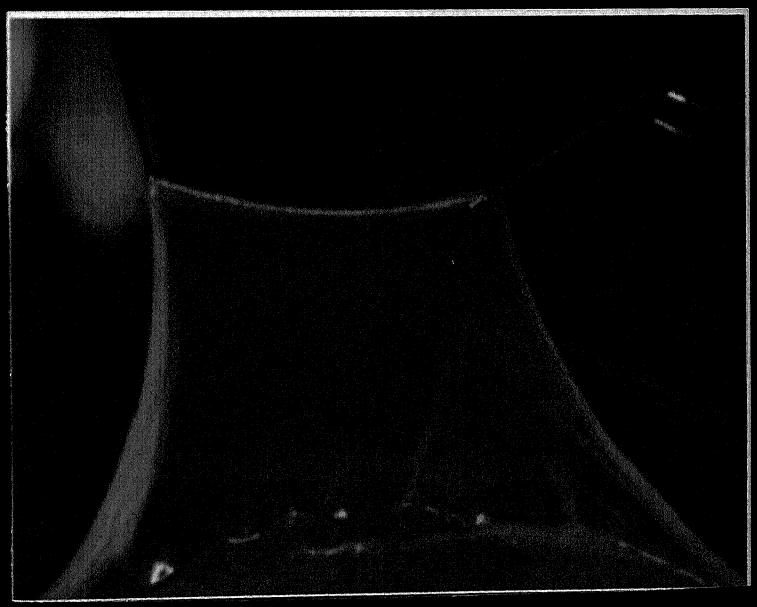
TABLE-10

RESULTS

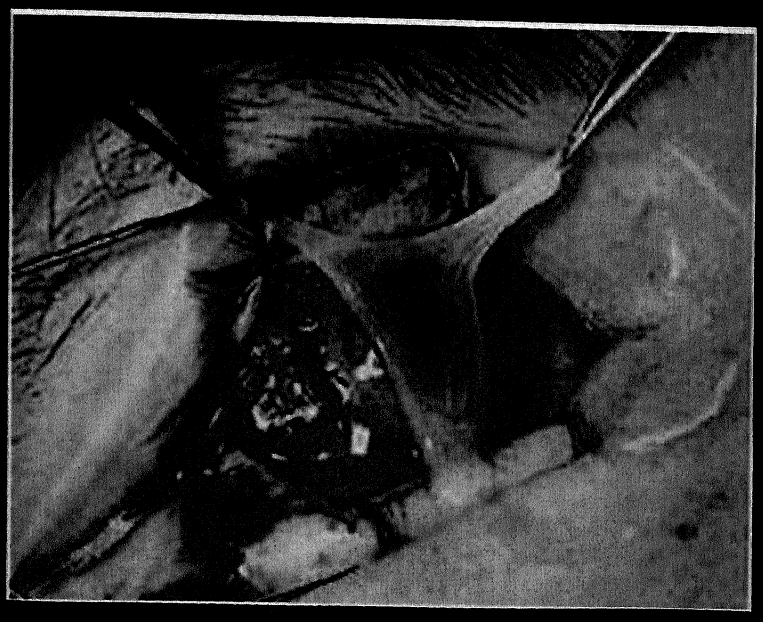
Indications for surgery	No. of cases	Success	Partial success	Failure
Non healing corneal ulcer	14	11 (78.5%)	03 (21.4%)	00 (00%)
Persistent epithelial defect	9	09 (100%)	00 (00%)	02 (22.2%)
Descemetocele	2	02 (100%)	00 (00%)	00 (00%)
Bullous keratopathy	4	03 (75.0%)	01 (25%)	00 (00%)
Pterygium	9	05 (55.5%)	03 (33.3%)	01 (11.11%)
Symblepheron	10	06 (50.0%)	02 (20%)	02 (20%)
Total	**48 (100)**	**36 (75%)**	**9 (18.75%)**	**03 (6.25%)**



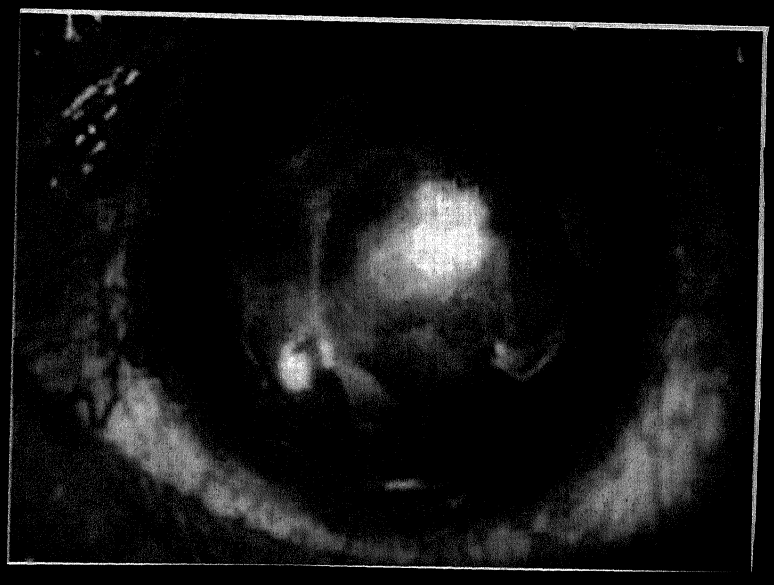
THE PLACENTAL MEMBRANES



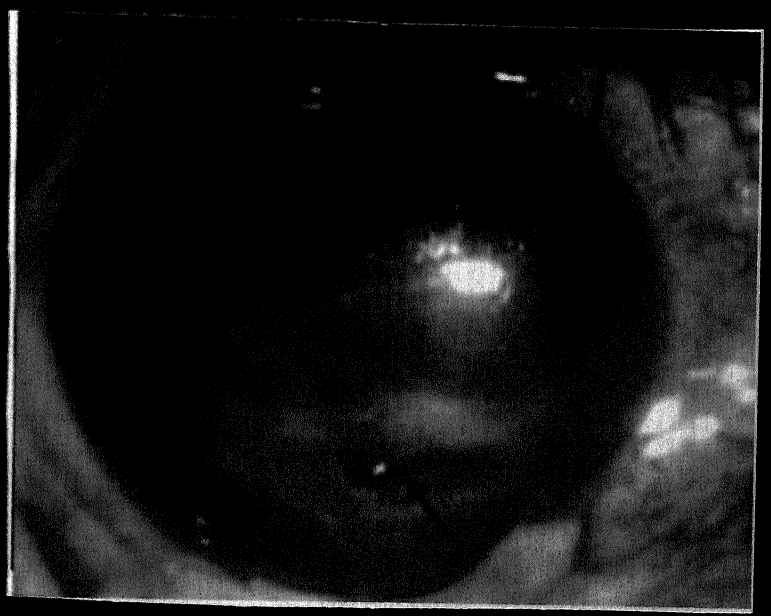
THE AMNIOTIC MEMBRANE



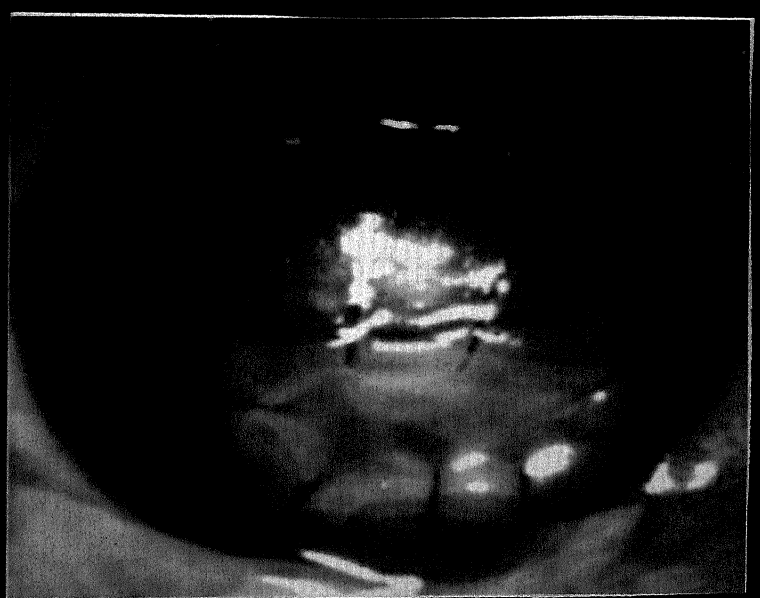
AMNIOTIC MEMBRANE TRANSPLANTATION



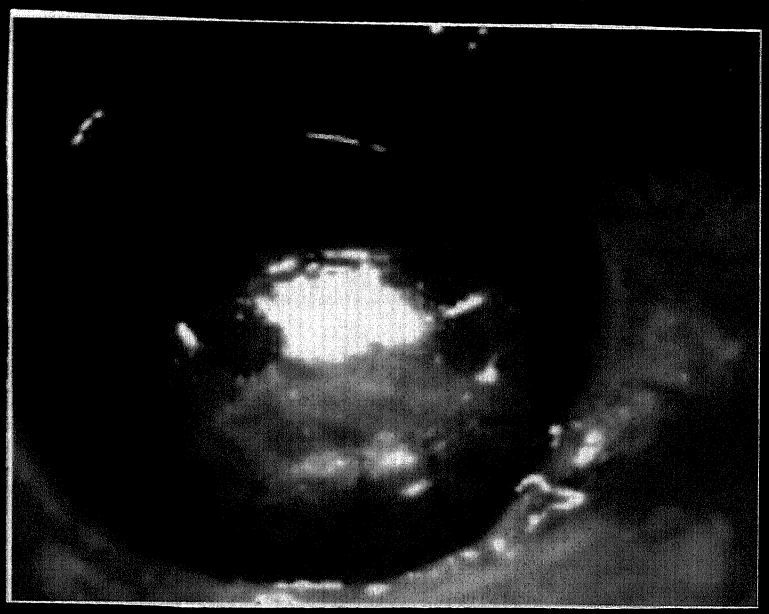
AMNIOTIC MEMBRANE TRANSPLANTATION
IN BULLOUS KERATOPATHY



THE PERFORATED CORNEAL ULCER



MULTILAYER AMNIOTIC MEMBRANE TRANSPLANTATION



MULTILAYER AMNIOTIC MEMBRANE TRANSPLANTATION WITH AN AMNIOTIC PATCH

FULLY RESTORED STROMAL THICKNESS WITH NO EPITHELIAL DEFECT

DISCUSSION

Discussion

Before amniotic membrane transplantation most eyes with nonhealing corneal ulcer had received multiple ocular surgeries. For exposure problems, patching and bandage contact lens had been tried in three eyes, ptosis had been induced by botulinum toxin injection in three eyes, partial tarsorrhaphy had been performed in two eyes, and conjunctival flap in one eye. For aqueous tear deficiency, punctal occlusion had been performed in eight eyes. Despite the above measures and use of frequent tear substitutes and lubricants and prophylactic antibiotics, all eyes presented with persistent or progressive epithelial defects and stromal ulceration of varying degrees The principal complaint was reduced vision in most of the patients and other complaints included annoying redness and ill defined irritation in some cases.

The conventional methods to treat persistent epithelial defect include the use of contact lens, conjunctival flap (Gundersen 1958; Insler et al. 1987) or tarsorrhaphy (Welch et al. 1988). Consistent with what has been reported by Lee (Lee et al. 1997) and Kruse (Kruse et al. 1999), our result also noted an overwhelming success of treating this indication.

In our study 11 out of 14 (78.5%) patients with Nonhealing corneal ulcer healed completely,while incomplete healing occurred in 3

cases.healing was not complete in these cases because 2 cases were of severe dry eye and one eye had corneal perforation of >2mm.healing was complete with corneal perforation of <1mm.

Similar study was conducted by **Rakowska E, Zagorski Z, Kardaszewska A, Durakiewicz D.(1999)** they presented their experience with the amniotic membrane transplantation in severe corneal diseases.Similarly **Kruse FE, Rohrschneider K, Volcker HE. (1999)** conducted a study evaluate the efficacy of multilayer amniotic membrane transplantation for reconstruction of corneal epithelium and stroma in the context of deep corneal ulcers. **Hong-Jeng Chen, Renato T F Pires, Scheffer C G Tseng (2000)** Conducted a study to evaluate whether amniotic membrane transplantation can be an effective alternative treatment for neurotrophic corneal ulcers. **Hanada K, Shimazaki J, Shimmura S, Tsubota K.(2001)** studied the efficacy of amniotic membrane transplantation in the treatment of deep corneal and scleral ulcers.**Pinnita Prabhasawat, Nattaporn Tesavibul, Wiwat Komolsuradej (2001)** Conducted a study to evaluate the efficacy of amniotic membrane transplantation (AMT) in persistent corneal epithelial defect with or without stromal thinning and corneal perforation. Study by **Zito E, Borderie V, Touzeau O, Bourcier T, Allouch C, Laroche L.**

in severe corneal epithelial diseases .Our success rate is almost similar to their results. The following table compares the indications and success rates of abovementioned studies.

Author	Indication	No. of cases	Success rate
Rakowska E, (1999)	Perforated corneal ulcers	9	77%
	Non-perforated ulcers	4	100%
Kruse FE, (1999)	Deep corneal ulcers	11	81%
Hong-Jeng Chen (2000)	Neurotrophic corneal ulcers	16	76.4%
Hanada K,(2001)	Deep corneal and scleral ulcers	11	72.7%
Pinnita Prabhasawat (2001)	Persistent corneal epithelial defect	10	80%
	Persistent corneal epithelial defect with stromal thinning	13	84.6%
	Corneal perforation	5	80%
Zito E, (2002)	Severe corneal epithelial diseases	14	75%
Our study	Non healing corneal ulcer	14	78.5%
	Persistent epithelial defect	9	100%

AMT not only promote epithelial healing,but also increases the corneal thickness in the case of descemetocele.Our study achieved success rate of 100%. Similarly **Solomon A, Meller D, Prabhasawat P, John T, Espana EM, Steuhl KP, Tseng SC.(2002)** Described the clinical outcome of amniotic membrane transplantation (AMT) for nontraumatic corneal perforations, descemetoceles, and deep ulcers. The following table shows result of their study.

Author	Indication	No. of cases	Success rate
Solomon A (2002)	corneal perforation, Descemetocele	34	82.3%
Our study	Descemetocele	2	100%

In bullous keratopathy, the cornea is edematous due to endothelial dysfunction. The corneal epithelium also becomes unhealthy and gets an irregular surface causing ocular pain and surface breakdown. This generally requires penetrating keratoplasty to relief the ocular discomfort and to improve vision. Lee (Lee et al. 1997) first reported that amniotic membrane can be used as an alternative to conjunctival graft to relief pain and recurrent erosion. ***We noted here amniotic membrane is an excellent alternative for countries like India where there is a shortage of corneal***

donors, to relief pain and reduce surface inflammation while the patient is on the waiting list for corneal transplantation.75% of our patients were relieved of pain and discomfort. One out of 4 was partially relieved due to unknown reason.

Although the mechanism remains unclear, we are intrigued by the finding that symptomatic relief persists even there remains marked edema and recurrent bullae several months following AMT. Similar study by **Mrukwa-Kominek E, Gierek-Ciaciura S, Rokita-Wala I, Szymkowiak M. (2002)** aimed to evaluate effectiveness of bullous keratopathy treatment using amniotic membrane transplantation. their study is compared with our study in the following table.

Author	Indication	No. of cases	Success rate
Mrukwa-Kominek E (2002)	Bullous keratopathy	18	100%
Our study	Bullous keratopathy	4	75%

It is believed that surgical trauma and subsequent postoperative inflammation activates subconjunctival fibroblasts, and the proliferation of fibroblasts and vascular cells, and deposition of extracellular matrix (ECM) proteins in turn contribute to the pterygium recurrence.

Alternatively, pterygium fibroblasts were reported to exhibit some characteristics of transformed cells such as hyperproliferation and overexpression of matrix metalloproteinases,which may partially explain the invasive nature of pterygium tissue.

As a natural basement membrane, amniotic membrane (AM) contains various matrix proteins which facilitate the adhesion, migration, differentiation, and prevention of apoptosis of epithelial cells. The AM is also capable of binding growth factors which may help to promote wound healing. Promotion of conjunctival epithelial wound healing and suppressing activation and ECM production by pterygium fibroblast are thought to be the major mechanisms by which an AM graft inhibits pterygium recurrence. Other possible mechanisms include inhibition of inflammation by inhibiting chemokines expression by fibroblasts and interleukin-1 expression by epithelial cells, inhibition of neovascularisation by inhibiting vascular endothelial cell growth, presence of anti-angiogenic/anti-inflammatory proteins, and protease inhibitors.

Shimazaki J, Shinozaki N, Tsubota K (1998) studied whether the treatment of recurrent pterygium associated with symblepharon requires both suppression of fibrosis and reconstruction of limbal barrier. To achieve this,they conducted the study in which human amniotic

membrane was transplanted and limbal autografts performed.**Gabric N, Mravicic I, Dekaris I, Karaman Z, Mitrovic S.(1999)** Sought to determine the efficacy of amniotic membrane transplantation (AMT) in the reconstruction of ocular surface. AMT was performed in 10 patients with recurrent pterygia .**Kobayashi A, Shirao Y, Segawa Y, Higashide T, Miwa S, Kawasaki K, Takata M, Tseng SC.(2001)** reported a case of successful management of pterygium with multi-layer amniotic membrane graft (AMG) in a young XP patient. These studies are summarized in the following table.

Authors	No. of patients	Success rate
Shimazaki J (1998)	4	100%
Gabric N (1999)	10	70%
Kobayashi A (2001)	1	100%
Our study	9	55.5%

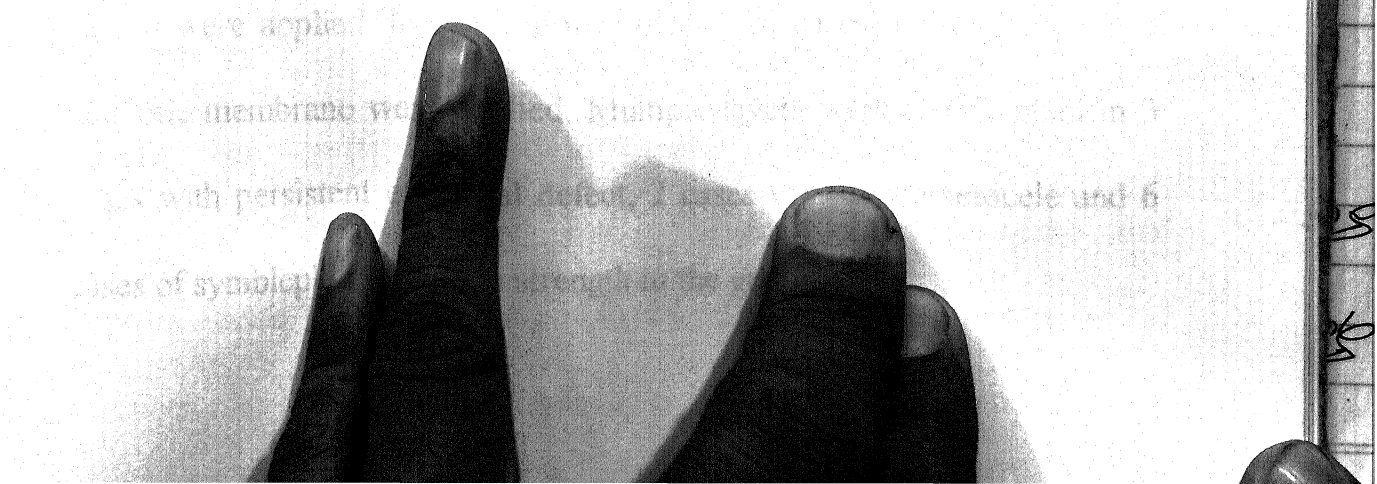
In our patients with symblepharon the transplantation of amniotic membrane was able to prevent the development of new symblepharon and helped to preserve vision in 60% of cases. Similar study conducted by **Tseng SC, Prabhasawat P, Lee SH (1997)** to determine whether preserved human amniotic membrane can be used to reconstruct the

conjunctival defect created during surgical removal of a large lesion or during symblepharon lysis.. Similarly **Zhou S, Chen J, Xu L, Lin J, Huang T (1999 Sep)** conducted a study to determine whether fresh human amniotic membrane can be used to reconstruct the conjunctival detect created during symblepharon lysis. **Honavar SG, Bansal AK, Sangwan VS, Rao GN (2000)** conducted a study to evaluate amniotic membrane transplantation (AMT) for ocular surface reconstruction in Stevens-Johnson syndrome (SJS).

Solomon A, Espana EM, Tseng SC. (2003) conducted a study to describe the clinical outcome of amniotic membrane transplantation (AMT) for fornix reconstruction in a variety of ocular surface disorders. These studies are summarized in the following table.

Author	Indication	No. of cases	Success rate
Tseng SC (1997)	Conjunctival defect reconstruction after surgical removal of a large lesion or during symblepharon lysis	16	68.7%
Zhou S (1999)	Conjunctival detect reconstruction after symblepharon lysis	42	88%
Honavar SG (2000)	For ocular surface reconstruction in Stevens-Johnson syndrome (SJS)	10	90%
Solomon A (2003)	Fornix reconstruction in a variety of ocular surface disorders	15	70.6%
Our study	Conjunctival detect reconstruction after symblepharon lysis	10	60%

SUMMARY AND CONCLUSION



Summary and Conclusion

In this study amniotic membrane transplantation was performed in patients with either non-healing corneal ulcer, persistent epithelial defect, descemetocele, bullous keratopathy, pterygium and symblepharon.

A total of 48 eyes of 48 patients underwent surgery over a period of 12 months, maximum number of cases were of non-healing corneal ulcer (14 cases) 29.16% followed by persistent epithelial defect, 9 cases (18.7%) and pterygium 9 cases (18.7%). Other indications included, descemetocele, 02 cases (4.44%), Bullous keratopathy 04 cases (8.33%) and symblepharon 10 cases (22.22%).

The study group included 34 male and 14 female. The average age of these patients was 46.6 years ranging from 12 years to 65 years.

Depending upon the size of lesion amniotic membrane graft of size 4mm to the size including whole of the cornea and 5-7 mm from the limbus were applied. In deep corneal ulcers (10 cases) multiple layers of amniotic membrane were applied. Multiple layers were also applied in 3 cases with persistent epithelial defect, 2 cases with descemetocele and 6 cases of symblepharon to give strength to the graft.

Although it was not uncommon to encounter minor problems such as detachment of membrane (9 cases), conjunctivitis (4 cases), corneal ulcer (2 cases), we did not note any sign of graft rejection and no patient became clinically worse than before the surgery in terms of visual acuity or inflammation. Only two cases developed corneal ulcer after complete healing of the lesion. This was due to rubbing of their eyes accidentally which was treated successfully on the same line of treatment for corneal ulcer.

Membrane was reapplied in cases where membrane detached within 1-2 days. The membrane did not detach further and lesion healed completely .Conjunctivitis was treated with topical antibiotics.

Based on the criteria given in the table-A, success, partial success and failure were obtained in 36 eyes (75%), 9 eyes (18.75%) and 3 eyes (6.25%) respectively. Details of the success rate in each group are shown in table-10.

In non healing corneal ulcer 11 out of 14 patients were rated as success and improved vision of approximately 2 Sneller's lines. Success rate was 100% in eyes with persistent epithelial defect and descemetocele. In painful bullous keratopathy, 3 out of 4 eyes achieved success rate of 75% and became pain free with stable corneal surface

after AMT. Cases with pterygium and symblepharon achieved success rate of 55.5% and 60% respectively.

In summary, this study has shown that the amniotic membrane can be used effectively to treat the ocular surface disorders.

From the study we can conclude that amniotic membrane transplantation in a safe, simple, inexpensive and effective for different ocular surface disorders. This technique has achieved success rate of 75% which is highly satisfactory.

BIBLIOGRAPHY

Bibliography

1. de Roth A. Plastic repair of conjunctival defects with fetal membrane. ***Arch Ophthalmol* 1940,23:522-5.**

2. Sorsby A, Symons HM. Amniotic membrane grafts in caustic burns of the eye. ***Br J Ophthalmol* 1946;30:337-45.**

3. Lavery FS. Lime burn of conjunctiva and cornea treated with amnioplastin graft. ***Trans Ophthalmol Soc U K. 1946;66:668.***

4. Sorsby A, Haythorne J, Reed H. Further experience with amniotic membrane grafts in caustic burns of the eye. ***Br J Ophthalmol 1947,31:409-18.***

5. Robson MC, Krizek TJ, Koss N, *et al*. Amniotic membranes as a temporary wound dressing. ***Surg Gynaecol Obstet 1973;136:904-906.***

6. Robson MC, Krizek TJ. The effect of human amniotic membranes on the bacterial population of infected rat burns. ***Ann Surg 1973;177:144-149.***

7. Colocho G, Graham WP, Greene AE, *et al*. Human amniotic membrane as a physiological wound dressing. ***Arch Surg 1974;109:370-37.***

8. Ninman C, Shoemaker P. Human amniotic membranes for burns. ***Am J Nurs 1975;75:1468-1469.***

9. Walker AB, Cooney DR, Allen JE. Use of fresh amnion as a burn dressing. ***J Paediatr Surg 1977;12:391-395.***

10. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. ***Am J Obstet Gynecol 1979;134:833-845.***

11. Bose B. Burn wound dressing with human amniotic membrane. ***Ann Roy Coll Surg Engl* 1979;*61:444-447.***

12. Twlford JD, Trelford-Sauder M. The amnion in surgery, past and present. ***Am J Obstet Gynecol 1979;134:833-845.***

13. Akle CA, Adinolfi M, Welsh KI, Leibowitz S, McColl 1. Immunogenicity of human amniotic epithelial cell after transplantation into volunteers, ***Lancet* 1981;2:1003-5.**

14. Thomson PD, Parks DH. Monitoring, banking, and clinical use of amnion as a burn wound dressing. ***Ann Plastic Surg 1981;7:354-356.***

15. Matthews RN, Faulk WP, Bennett JP. A review of the role of amniotic membranes in surgical practice. ***Obstet Gynaecol Ann 1982;11:31-58.***

16. Kim JC and Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. ***Cornea 1995;14:473-484.***

17. Lee HS and Kim JC. Effect of amniotic fluid in corneal sensitivity and nerve regeneration after excimer laser ablation. ***Cornea 1996;15:517-524.***

18. Tsubota K et al. Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. ***Am J Ohthalmol 1996;122:38.52.***

19. Shimazaki et al. Amniotic membrane transplantation for ocular surface reconstruction in patients with chemical and thermal burns. ***Ophthalmology 1997;104:2068-2076.***

20. Prabhasawat P, Barton K, Burkett G, Tseng SGC. Comparison of conjunctival autografts, amniotic membrane grafts and primary closure for pterygium excision. ***Ophthalmology 1997;104:974-985.***

21. Choi YS, Kim JY, Wee WR et al. Application of amniotic membrane on corneal wound healing after excimer laser PRK. ***Invest Ophthalmol Vis Sci 1997;38:S536.***

22. Wang M, Prabhasawat P et al. Corneal haze is reduced by amniotic membrane matrix in excimer laser photoablation in rabbits. ***Invest Ophthalmol Vis Sci 1997;38:S405.***

23. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. ***Am J Ophthalmol. 1997 Mar;123(3):303-12.***

24. Tseng SC, Prabhasawat P, Lee SH. Amniotic membrane transplantation for conjunctival surface reconstruction. ***Am J Ophthalmol 1997 Dec;124(6):765-74.***

25. Panda A. Yet another surgery (amniotic membrane grafting) in 16 eyes of ocular surface disorders. ***Delhi Ophthalmological Society Times 1998;3:5-7.***

26. Panda A, Pushkar N. A new approach to ocular surface reconstruction. ***Delhi Ophthalmological Society Times 1998;6:29-31.***

27. Shimazaki J, Shinozaki N, Tsubota K. Transplantation of amniotic membrane and limbal autograft for patients with recurrent pterygium associated with symblepharon. ***Br J Ophthalmol 1998 Mar;82(3):235-40.***

28. Tseng SC, Prabhasawat P, Barton K, Gray T, Meller D. Amniotic membrane transplantation with or without limbal allografts for corneal surface reconstruction in patients with limbal stem cell deficiency. ***Arch Ophthalmol. 1998 Apr;116(4):431-41.***

29. Meller D, Tseng SC. Reconstruction of the conjunctival and corneal surface. Transplantation of amnionic membrane ***Ophthalmologe 1998 Dec;95(12):805-13.***

30. Gris O, Guell JL, Lopez-Navidad A, Caballero F, Del Campo Z. Application of the amniotic membrane in ocular surface pathology. ***Ann Transplant. 1999;4(3-4):82-4.***

31. Gabric N, Mravicic I, Dekaris I, Karaman Z, Mitrovic S. Human amniotic membrane in the reconstruction of the ocular surface. ***Doc Ophthalmol. 1999;98(3):273-83.***

32. Rodriguez-Ares MT, Tourino R, Capeans C, Sanchez-Salorio M. Repair of scleral perforation with preserved scleral and amniotic membrane in Marfan's syndrome .***Ophthalmic Surg Lasers. 1999 Jun;30(6):485-7.***

33. Rakowska E, Zagorski Z, Kardaszewska A, Durakiewicz D. Application of amniotic membrane transplantation in severe corneal diseases ***Klin Oczna. 1999;101(6):417-21.***

34. Kruse FE, Rohrschneider K, Volcker HE. Multilayer amniotic membrane transplantation for reconstruction of deep corneal ulcers. ***Ophthalmology. 1999 Aug;106(8):1504-10; discussion 1511.***

35. Zhou S, Chen J, Xu L, Lin J, Huang T. Fresh amniotic membrane transplantation for conjunctival surface reconstruction ***Yan Ke Xue Bao 1999 Sep;15(3):169-73.***

36. Meller D, Tseng SC. Amniotic membrane transplantation with or without limbal allografts in corneal surface reconstruction in limbal deficiency ***Ophthalmologe. 2000 Feb;97(2):100-7.***

37. Pires RT, Chokshi A, Tseng SC. Amniotic membrane transplantation or conjunctival limbal autograft for limbal stem cell deficiency induced by 5-fluorouracil in glaucoma surgeries. ***Cornea. 2000 May;19(3):284-7.***

38. Meller D, Pires RT, Mack RJ, Figueiredo F, Heiligenhaus A, Park WC, Prabhasawat P, John T, McLeod SD, Steuhl KP, Tseng SC. Amniotic membrane transplantation for acute chemical or thermal burns. ***Ophthalmology 2000 May;107(5):980-9; discussion 990.***

39. Honavar SG, Bansal AK, Sangwan VS, Rao GN. Amniotic membrane transplantation for ocular surface reconstruction in

Stevens-Johnson syndrome. ***Ophthalmology 2000 May;107(5):975-9.***

40. Hong-Jeng Chen[a], Renato T F Pires[a], Scheffer C G Tseng[a] Amniotic membrane transplantation for severe neurotrophic ***corneal ulcers Br J Ophthalmol 2000;84:826-833 (August).***

41. Meller D, Maskin SL, Pires RT, Tseng SC. Amniotic membrane transplantation for symptomatic conjunctivochalasis refractory to medical treatments. ***Cornea. 2000 Nov;19(6):796-803.***

42. Hanada K, Shimazaki J, Shimmura S, Tsubota K. Multilayered amniotic membrane transplantation for severe ulceration of the cornea and sclera. ***Am J Ophthalmol 2001 Mar;131(3):324-31.***

43. Prabhasawat P, Kosrirukvongs P, Booranapong W, Vajaradul Y.Amniotic membrane transplantation for ocular surface reconstruction. ***J Med Assoc Thai 2001 May;84(5):705-18.***

44. Dekaris I, Gabric N, Mravicic I, Karaman Z, Katusic J, Lazic R, Spoljaric N. Multilayer vs. monolayer amniotic membrane transplantation for deep corneal ulcer treatment. ***Coll Antropol 2001;25 Suppl:23-8.***

45. Duchesne B, Tahi H, Galand A. Use of human fibrin glue and amniotic membrane transplant in corneal perforation.***Cornea. 2001 Mar;20(2):230-2.***

46. Hanada K, Shimazaki J, Shimmura S, Tsubota K. Multilayered amniotic membrane transplantation for severe ulceration of the cornea and sclera. ***Am J Ophthalmol. 2001 Mar;131(3):324-31.***

47. Anderson DF, Ellies P, Pires RT, Tseng SC. Amniotic membrane transplantation for partial limbal stem cell deficiency. ***Br J Ophthalmol. 2001 May;85(5):567-75.***

48. Letko E, Stechschulte SU, Kenyon KR, Sadeq N, Romero TR, Samson CM, Nguyen QD, Harper SL, Primack JD, Azar DT, Gruterich M, Dohlman CH, Baltatzis S, Foster CS. Amniotic membrane inlay and overlay grafting for corneal epithelial defects and stromal ulcers. ***Arch Ophthalmol. 2001 May;119(5):659-63.***

49. Kubo M, Sonoda Y, Muramatsu R, Usui M. Immunogenicity of human amniotic membrane in experimental xenotransplantation. ***Invest Ophthalmol Vis Sci. 2001 Jun;42(7):1539-46.***

50. Adds PJ, Hunt CJ, Dart JK. Amniotic membrane grafts, "fresh" or frozen? A clinical and in vitro comparison. ***Br J Ophthalmol. 2001 Aug;85(8):905-7.***

51. Tseng SC. Amniotic membrane transplantation for ocular surface reconstruction. ***Biosci Rep. 2001 Aug;21(4):481-9.***

52. Ferreira De Souza R, Hofmann-Rummelt C, Kruse FE, Seitz B. Multilayer amniotic membrane transplantation for corneal ulcers not treatable by conventional therapy - a prospective study of the status of cornea and graft during follow-up] .***Klin Monatsbl Augenheilkd. 2001 Aug;218(8):528-34.***

53. Kobayashi A, Shirao Y, Segawa Y, Higashide T, Miwa S, Kawasaki K, Takata M, Tseng SC. Multi-layer amniotic membrane graft for pterygium in a patient with xeroderma pigmentosum. ***Jpn J Ophthalmol 2001 Sep-Oct;45(5):496-8.***

54. Kruse FE, Meller D. Amniotic membrane transplantation for reconstruction of the ocular surface .***Ophthalmologe 2001 Sep;98(9):801-10.***

55. Moore JE, Dua HS, Page AB, Irvine AD, Archer DB. Ocular surface reconstruction in LOGIC syndrome by amniotic membrane transplantation. ***Cornea 2001 Oct;20(7):753-6.***

56. Pinnita Prabhasawat[a], Nattaporn Tesavibul[b], Wiwat Komolsuradej[c] Single and multilayer amniotic membrane transplantation for persistent corneal epithelial defect with and without stromal

thinning and perforation .***Br J Ophthalmol 2001;85:1455-1463 (December).***

57. Toczolowski J, Klonowski P, Pozarowska D, Wozniak F, Krzyzanowski A, Semczuk-Sikora A, Semczuk M. Use of amnion in the treatment of anterior segment diseases of the eye. ***Klin Oczna 2001;103(2-3):91-4.***

58. Grueterich M, Espana E, Tseng SC. Connexin 43 expression and proliferation of human limbal epithelium on intact and denuded amniotic membrane. ***Invest Ophthalmol Vis Sci. 2002 Jan;43(1):63-71.***

59. Ucakhan OO, Koklu G, Firat E. Nonpreserved human amniotic membrane transplantation in acute and chronic chemical eye injuries. ***Cornea 2002 Mar;21(2):169-72.***

60. Mrukwa-Kominek E, Gierek-Ciaciura S, Rokita-Wala I, Szymkowiak M. Use of amniotic membrane transplantation for treating bullous keratopathy. ***Klin Oczna 2002;104(1):41-6.***

61. Solomon A, Meller D, Prabhasawat P, John T, Espana EM, Steuhl KP, Tseng SC. Amniotic membrane grafts for nontraumatic corneal perforations, descemetoceles, and deep ulcers. ***Ophthalmology 2002 Apr;109(4):694-703.***

62. Meller D, Dabul V, Tseng SC. Expansion of conjunctival epithelial progenitor cells on amniotic membrane. ***Exp Eye Res. 2002 Apr;74(4):537-45.***

63. Meller D, Pires RT, Tseng SC. Ex vivo preservation and expansion of human limbal epithelial stem cells on amniotic membrane cultures. ***Br J Ophthalmol. 2002 Apr;86(4):463-71.***

64. Pan Z, Zhang W, Wu Y, Sun B. Transplantation of corneal stem cells cultured on amniotic membrane for corneal burn: experimental and clinical study. ***Chin Med J (Engl). 2002 May;115(5):767-9.***

65. Grueterich M, Tseng SC. Human limbal progenitor cells expanded on intact amniotic membrane ex vivo. ***Arch Ophthalmol. 2002 Jun;120(6):783-90.***

66. Shimazaki J, Aiba M, Goto E, Kato N, Shimmura S, Tsubota K. Transplantation of human limbal epithelium cultivated on amniotic membrane for the treatment of severe ocular surface disorders. ***Ophthalmology. 2002 Jul;109(7):1285-90.***

67. Stoiber J, Muss WH, Pohla-Gubo G, Ruckhofer J, Grabner G. Histopathology of human corneas after amniotic membrane and limbal stem cell transplantation for severe chemical burn. ***Cornea. 2002 Jul;21(5):482-9.***

68. Stoiber J, Ruckhofer J, Muss W, Grabner G. Amniotic membrane transplantation with limbal stem cell transplantation as a combined procedure for corneal surface reconstruction after severe thermal or chemical burns.***Ophthalmologe. 2002 Nov;99(11):839-48.***

69. Zito E, Borderie V, Touzeau O, Bourcier T, Allouch C, Laroche L.Amniotic membrane transplantation in severe corneal epithelial diseases. Preliminary results. ***J Fr Ophtalmol. 2002 Nov;25(9):879-88.***

70. Nakamura T, Koizumi N, Tsuzuki M, Inoki K, Sano Y, Sotozono C, Kinoshita S. Successful regrafting of cultivated corneal epithelium using amniotic membrane as a carrier in severe ocular surface disease. ***Cornea. 2003 Jan;22(1):70-1.***

71. Solomon A, Espana EM, Tseng SC. Amniotic membrane transplantation for reconstruction of the conjunctival fornices. ***Ophthalmology 2003 Jan;110(1):93-100.***

72. Barabino S, Rolando M. Amniotic membrane transplantation elicits goblet cell repopulation after conjunctival reconstruction in a case of severe ocular cicatricial pemphigoid. ***Acta Ophthalmol Scand 2003 Feb;81(1):68-71.***

73. Su WY, Chang SW, Huang SF. Amniotic membrane transplantation for corneal perforation related to vitamin A deficiency. ***Ophthalmic Surg Lasers Imaging. 2003 Mar-Apr;34(2):140-4.***

74. Barabino S, Rolando M, Bentivoglio G, Mingari C, Zanardi S, Bellomo R, Calabria G. Role of amniotic membrane transplantation for conjunctival reconstruction in ocular-cicatricial pemphigoid. ***Ophthalmology 2003 Mar;110(3):474-80.***

75. Sangwan VS, Vemuganti GK, Iftekhar G, Bansal AK, Rao GN. Use of autologous cultured limbal and conjunctival epithelium in a patient with severe bilateral ocular surface disease induced by acid injury: a case report of unique application. ***Cornea. 2003 Jul;22(5):478-81.***

76. Meallet MA, Espana EM, Grueterich M, Ti SE, Goto E, Tseng SC. Amniotic membrane transplantation with conjunctival limbal autograft for total limbal stem cell deficiency. ***Ophthalmology. 2003 Aug;110(8):1585-92.***

77. ska H, Czajka M. Transplantation of amniotic membrane for patients with bullous keratopathy and chemical and thermal burn. ***Klin Oczna. 2003;105(1-2):41-5.***

78. T. Nakamura, K.-I. Endo, L. J. Cooper, N. J. Fullwood, N. Tanifuji, M. Tsuzuki, N. Koizumi, T. Inatomi, Y. Sano, and S. Kinoshita.The Successful Culture and Autologous Transplantation of Rabbit Oral Mucosal Epithelial Cells on Amniotic Membrane. ***Invest. Ophthalmol. Vis. Sci., January 1, 2003; 44(1): 106 - 116.***